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Endothelium-derived Hyperpolarizing Factor

A Cousin to Nitric Oxide and Prostacyclin

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There is now strong evidence that an endothelial mechanism, other than nitric oxide or prostacyclin, exists for dilating arteries and arterioles. This third pathway has been named *endothelium-derived hyperpolarizing factor* (EDHF) and should not be confused with endothelium-derived relaxing factor, which is nitric oxide. Currently, there are several ideas for the mechanism of EDHF, which may vary among vessels of different organs and species. During some pathologic states, EDHF can be up-regulated. This up-regulation often occurs as the dilator effects of endothelium-derived nitric oxide are suppressed. The up-regulated EDHF may serve in a protective capacity to help maintain blood flow to organs and tissues during these stressful states. Many anesthetics attenuate the dilator actions of EDHF; however, the full clinical implications of this anesthetic-related attenuation are not known. Like its cousins, nitric oxide and prostacyclin, EDHF is an important regulator of blood flow and should prove to be an important clinical consideration as we gain more knowledge of its mechanisms of action.

THE endothelium consists of a single layer of cells on the luminal surface of all vessels of the vascular system (fig. 1A). Initially, it was thought that the endothelium functioned only as an antithrombotic surface to prevent aggregation of blood products and as a barrier to prevent exchange of certain molecules between plasma and tissue. However, in the 1980s, it became apparent that the endothelium also regulates the contractile state of vascular smooth muscle. Activation of receptors on endothelium or mechanical forces exerted on endothelial cells releases factors that contract (thromboxane and endothelin) or relax (nitric oxide and prostacyclin) vascular smooth muscle.[1,2] The discovery that the endothelium releases these relaxing and contracting factors sparked new and exciting investigations into circulatory control.

The single most significant contribution reported an "endothelium-derived relaxing factor" that was later identified as nitric oxide.[3–5] On stimulation of endothelial receptors or deformation of the endothelium by mechanical forces, endothelial nitric oxide synthase (eNOS) can be activated through increases of intracellular Ca^{2+}, stimulation of protein kinases to synthesize nitric oxide, or both (fig. 1B). The newly synthesized nitric oxide diffuses from the endothelium to the vascular smooth muscle, where it stimulates soluble guanylyl cyclase to generate cyclic guanosine monophosphate. Through activation of protein kinase G, cyclic guanosine monophosphate relaxes the vascular smooth muscle by a number of mechanisms, which include decreasing cytoplasmic free Ca^{2+}, decreasing sensitivity to Ca^{2+}, or both. In addition to generation of cyclic guanosine monophosphate, other mechanisms of dilation for nitric oxide have been reported.[6,7] In a similar manner, prostacyclin (PGI_2) can be synthesized by cyclooxygenase (COX) and released from the endothelium; however, PGI_2 elicits smooth muscle relaxation by stimulating adenylyl cyclase and generation of cyclic adenosine monophosphate[2] (fig. 1B).

In the late 1980s and early 1990s, evidence began to emerge that there was at least one additional endothelium-dependent process responsible for relaxing vascular smooth muscle. The process was characterized by an essential hyperpolarization of the vascular smooth muscle and could be blocked by inhibitors of potassium channels. The process became known as *endothelium-derived hyperpolarizing factor* (EDHF).[8–10] Unlike its predecessor, endothelium-derived relaxing factor, which required approximately 6 yr before conclusively being identified as nitric oxide, the mechanism of EDHF remains controversial even today, more than 15 yr after first being described. The elusive nature of EDHF is likely due to the complexity of the mechanisms and the fact that there are several EDHFs or mechanisms by which the endothelium can hyperpolarize vascular smooth muscle.

Endothelium-derived hyperpolarizing factor is defined as a dilator process that (1) requires endothelium; (2) is distinct from both endothelium-derived nitric oxide or COX metabolites (*i.e.*, PGI_2); (3) dilates by hyperpolarizing the vascular smooth muscle; and (4) involves potassium channel activation, most often calcium-activated

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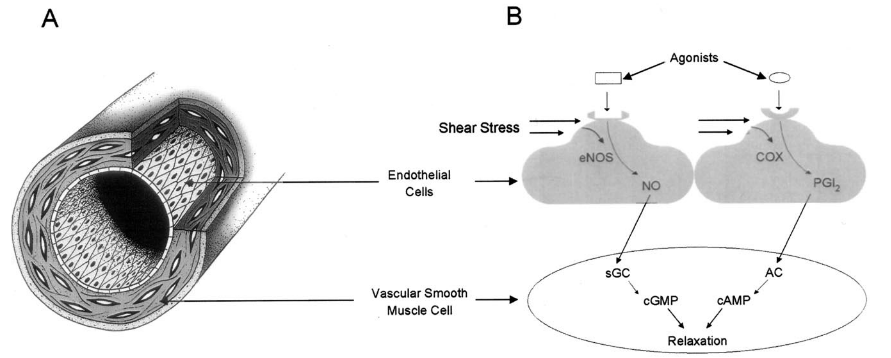

Fig. 1. (*A*) Diagram of an artery showing endothelial and vascular smooth muscle cells. (*B*) Mechanisms of endothelial-mediated dilations through nitric oxide (NO) and prostacyclin (PGI_2). AC = adenylyl cyclase; cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate; COX = cyclooxygenase; eNOS = endothelial nitric oxide synthase; sGC = soluble guanylyl cyclase.

potassium channels (K_{Ca}).[11] Definitions of EDHF vary. Some investigators reserve the acronym *EDHF* for that factor whose dilation or smooth muscle hyperpolarization fits not only the above criteria but is also blocked by a combination of charybdotoxin and apamin, but not iberiotoxin and apamin (table 1).[12] EDHF dilations can be elicited by a number of endothelial agonists, shear stress, or pulsatile stretch.[11,13–21]

The inclusion of the term *factor* in the name *endothelium-derived hyperpolarizing factor* may not be appropriate in some vessels where a process, rather than a single transferable factor, is responsible for the dilation. Therefore, a more appropriate name to describe these dilations where a transferable factor is not involved would be *endothelium-dependent hyperpolarization*.[22,23] This review retains the traditional name and refers to the dilation as EDHF, independent of whether a factor is involved.

Currently, our understanding of EDHF is limited. Most studies to date have concentrated on understanding the mechanism, whereas its role in regulating and coordinating blood flow has received much less attention. Our limited knowledge precludes the medical community from effectively manipulating EDHF in the clinical setting. However, an evolving understanding of EDHF during normal and pathologic states predicts that it will become an important therapeutic target.[22,23] In the future, EDHF will likely be manipulated to aid in regulating blood pressure and/or to selectively regulate perfusion to vital organs such as the brain, heart, and kidney. The purpose of this review is to acquaint the anesthesiologist with EDHF, its role as a dilator mechanism, and the potential clinical implications that may be derived from understanding it and its role in circulatory control. In this way, the anesthesiologist will be able to be follow the field as it evolves and matures, with the potential to eventually use EDHF as a therapeutic target in the operating room and intensive care unit. Just as endothelium-derived nitric oxide has become an important consideration for the practice of anesthesiology, EDHF is likely to follow as we gain knowledge of its mechanisms of action, its physiologic role, and its regulation by anesthetics.

Table 1. K Channels Relevant to EDHF Studies

K Channel	Inhibitor	Note
BK_{Ca}*	Iberiotoxin Charybdotoxin Tetraethylammonium	Tetraethylammonium is selective up to 1 mM; charybdotoxin also inhibits some K_v channels and IK_{Ca}.
IK_{Ca}†	Charybdotoxin TRAM-34	Charybdotoxin also inhibits some K_v channels and BK_{Ca}.
SK_{Ca}‡	Apamin Dequalinium	
K_{ir}§	Ba^{2+}‖	Ba^{2+} is concentration selective up to 100 μM.

* Large- or big-conductance Ca-activated K channels. † Intermediate-conductance Ca-activated K channels. ‡ Small-conductance Ca-activated K channels. § Inwardly rectifying K channels. ‖ Barium ion.

EDHF = endothelium-derived hyperpolarizing factor; TRAM-34 = (1-[(2-chlorophenyl) diphenylmethyl]-1*H*-pyrazole).

EDHF Dilations in Isolated Arteries and Arterioles

Vascular reactivity is often studied in isolated arteries and arterioles. The two most common methods are to directly measure isometric force generation of the vessel or to measure diameter changes of the vessel. For the studies involving force measurement, a decrease in force or relaxation of the smooth muscle is equivalent to vessel dilation. When endothelial receptors are exposed to certain agonists, a dose-dependent dilation or relaxation of the vessel occurs (figs. 2A–C, solid lines). Depending on the vessel size and vessel type, endothelium-dependent dilations can be elicited by a number of agonists, including acetylcholine, bradykinin, substance P, adenosine triphosphate (ATP), adenosine diphosphate, uridine triphosphate, vasopressin, and histamine. The endothelial-mediated dilations elicited by the above agonists often involve the production and release of nitric oxide, PGI_2, or a combination of both (fig. 1).

After complete inhibition of nitric oxide synthase (NOS) and COX to inhibit nitric oxide and PGI_2 production, respectively, residual dilation often remains (figs. 2A–C, dashed lines). Figures 2A–C depict models of the residual dilations based on published results.[24–26] The difference between the original dilatory curve (solid

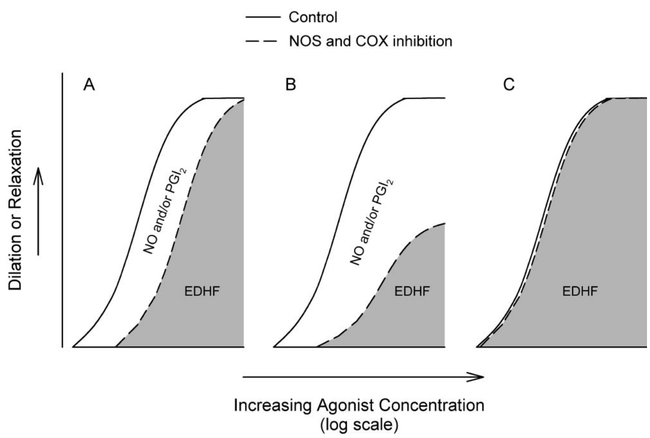

Fig. 2. Effects of increasing agonist concentration on endothelial-mediated dilations during control conditions (*solid lines*) and after inhibition of nitric oxide (NO) and prostacyclin (PGI_2) (*dashed lines*). Three responses that often occur in arteries after the inhibition are shown in *A*, *B*, and *C*.[24–26] COX = cyclooxygenase; EDHF = endothelium-derived hyperpolarizing factor; NOS = nitric oxide synthase.

lines) and the curve after inhibition of NOS and COX (dashed lines) can be considered the portion of the dilation that is attributable to nitric oxide and COX metabolites. The residual dilatory curve after NOS and COX inhibition may be shifted to the right[24,25] (fig. 2A), shifted to the right with a suppression in the maximum response[26] (fig. 2B), or identical to the original curve before NOS and COX inhibition[24] (fig. 2C). The residual dilations after inhibition of NOS and COX in figure 2 (dashed lines) are generally considered to be the EDHF component.

Endothelium-derived hyperpolarizing factor–mediated dilations hyperpolarize the vascular smooth muscle by 15–30 mV.[24,25,27] Note that smooth muscle hyperpolarization is not unique to EDHF. Nitric oxide and PGI_2 can also hyperpolarize vascular smooth muscle to varying degrees by activating potassium channels. The smooth muscle hyperpolarization elicits relaxation (or dilation) by decreasing the concentration of cytoplasmic free Ca^{2+} through closure of voltage-operated Ca channels in the smooth muscle cell membrane. The cytoplasmic concentration of free Ca^{2+} is a major determinant of the contractile state of smooth muscle. In general, increases in Ca^{2+} concentration produce contractions, whereas decreases in Ca^{2+} relax the smooth muscle cell. In addition to regulating Ca^{2+} concentrations, the sensitivity to cytoplasmic Ca^{2+} can be regulated by kinases and phosphatases to alter the contractile state of vascular smooth muscle. However, it is not known whether EDHF affects vascular smooth muscle sensitivity to Ca^{2+}.

One of the defining characteristics of EDHF is that it is inhibited by blocking potassium channels. A combination of potassium channel blockers is often required to effectively block the response. Table 1 shows the potassium channels most relevant to EDHF studies and their inhibitors. Depending on the mechanistic model (see "Mechanisms of EDHF Dilations"), the location of the potassium channels involved with the EDHF response can be on the endothelium, vascular smooth muscle, or both.

Physiologic Role and Diversity

Because of the limited knowledge that we have regarding EDHF, its role remains to be fully elucidated. Nevertheless, one observation that may provide a significant clue as to its physiologic role is that EDHF seems to be more prominent in smaller arteries and arterioles than in larger arteries. This observation has been made in a number of vascular beds, including those from the mesenteric, cerebral, ear, and stomach.[24,28–32] In fact, control of vessel diameter in these smaller arteries and arterioles by EDHF may be more important than endothelium-derived nitric oxide. For example, proceeding from larger to smaller arteries and arterioles, the relative importance of EDHF increased while that of endothelium-derived nitric oxide decreased.[24,32] Because of the fundamental role of these smaller vessels in the control of vascular resistance, it would therefore seem that EDHF plays a significant role in the regulation of vascular resistance and thus in the control of blood flow during normal physiologic conditions. Although there are uncertainties regarding the relative contributions of endothelium-derived nitric oxide and EDHF, it is possible that EDHF may be the more important of the two in normal regulation of blood flow in some organs of the body.

Another physiologic role for EDHF may be in conducted dilations of arterioles. When an artery or arteriole is stimulated to dilate at a focal site, the dilation can be conducted several millimeters upstream and downstream from the foci. Micropipette application of certain substances onto the surface of arterioles induces both a local vasomotor response as well as a response that is propagated along the vessel, both upstream and downstream to the application. This phenomenon is termed *conducted vasomotor response*. This conducted dilation is involved with the spatial and temporal regulation of blood flow within a microvascular network. For example, optimum blood flow control in the exercising muscle requires an overall coordination of vascular resistances. Without a functional conducted dilator response, areas within the microvascular network could be at risk for insufficient delivery of oxygen during times of maximum exercise. The conducted dilation is an important aspect of this coordinated response and is required to maximize blood flow control.[33]

In the intact hamster cheek pouch or cremaster microcirculatory beds, application of acetylcholine at a focal site produced a conducted dilation approximately 1 mm upstream of the application.[34,35] Inhibition of COX or NOS had little or no effect on the conducted dilation.

However, inhibitors of P-450 epoxygenase, which inhibit EDHF dilations in some vessels, or blockers of K_{Ca} significantly suppressed the conducted vasodilation to acetylcholine.[34,35] Thus, EDHF seems to have a major role in conducted dilations and the coordination of vascular resistances within the microcirculation.[34,35]

If EDHF has a widespread physiologic role, it follows that it should be found in a number of vessel types. Indeed, evidence for EDHF exists in a wide diversity of arteries from mammals. In humans, EDHF or EDHF-like dilations have been described in coronary arteries and arterioles,[36,37] cerebral arteries,[38] renal arteries,[39] interlobar arteries,[40] penile resistance arteries,[41] internal mammary arteries,[42,43] subcutaneous resistance arteries,[44,45] radial arteries,[46] gastroepiploic arteries,[29] mesenteric arteries,[47] and omental arteries.[48,49] The widespread existence of EDHF provides evidence for a significant physiologic role in the regulation of blood flow.

Most studies of EDHF have used isolated arteries and arterioles, *i.e.*, *ex vivo* vessels studied in a dish or organ bath as mentioned previously. If EDHF is an important regulator of blood flow, it must also be functional in intact animals. EDHF or EDHF-like dilations have been demonstrated *in vivo* in canine coronary and kidney arterioles,[50–52] hamster cremaster and cheek pouch arterioles,[34,35] rat cremaster arterioles,[53] and rat mesenteric, hind limb, and sciatic nerve circulations.[54,55] In humans, forearm blood flow shows an EDHF-like dilation with the administration of bradykinin or acetylcholine.[56–58] Therefore, EDHF has been reported in a wide diversity of vascular beds and in virtually all mammalian species studied, most important of which is the human.

Hormones seem to alter the EDHF response. Estrogen, the most studied of these hormones, seems to up-regulate EDHF in peripheral vessels and down-regulate EDHF in the cerebral circulation. Relaxation responses in the perfused mesenteric bed in male and female rats were similar. The addition of a NOS inhibitor attenuated the relaxation response in males but had no effect in females.[59,60] The authors suggested that EDHF is functionally more important in females than males in the mesenteric circulation. In mesenteric arteries from female rats, EDHF dilations were attenuated after ovariectomy when compared with intact rats.[61] Supplementing ovariectomized rats with estrogen rescued the EDHF response. Similarly, the EDHF response was reduced during diestrus, a time of low estrogen, when compared with estrus controls. Interestingly, supplementing male rats with 17β-estradiol or the phytoestrogen daidzein up-regulated EDHF in the aorta.[62]

In contrast to the peripheral circulation, EDHF in cerebral arteries and arterioles is down-regulated by estrogen. The EDHF response in isolated rat middle cerebral arteries was dramatically reduced in female rats as compared with male rats. The EDHF response in ovariectomized females was identical to the response in male rats and could be reversed by estrogen replacement.[63,64] *In vivo* studies of pial arterioles in intact female rats, ovariectomized rats, and ovariectomized rats with estrogen replacement came to the same conclusion that estrogen down-regulates EDHF.[65] The up-regulation of EDHF after ovariectomy involves gap junctions[66] but does not seem to be related to a repressed endothelial NOS-derived nitric oxide–generating function.[67]

Pregnancy is characterized by an increased sensitivity to endothelial dependent dilators.[68,69] A number of studies provide strong evidence that an up-regulated EDHF may be a major component to the vascular adaptations to pregnancy.[68–73] Interestingly, in preeclamptic patients, EDHF may not be up-regulated during pregnancy.[73,74] It is not known whether this failure to up-regulate EDHF is the cause or the result of the pathologic condition.

The male sex hormone, testosterone, seems to increase vascular tone or contractile state in cerebral arteries by suppressing EDHF.[75] An interesting twist involving this study is that the reported EDHF was not agonist induced but was present in the resting pressurized state.

Cortisol may also alter EDHF dilations. Exposure of the porcine coronary artery to cortisol for 24 h, but not 30 min, up-regulated EDHF-mediated dilations.[76] Concomitant with the up-regulation of the EDHF response, cytochrome P-450 2C expression was increased. The authors suggested that chronic cortisol exposure potentiates the EDHF response by up-regulating an epoxygenase that converts arachidonic acid to epoxyeicosatrienoic acids (EETs) that are putative EDHFs.[76]

Mechanisms of EDHF Dilations

Although much of the mechanism is controversial, there is agreement that an increase of free Ca^{2+} in endothelial cells is an initial step required for EDHF dilations (fig. 3). Interestingly, it is this Ca^{2+} increase in endothelial cells that ultimately leads to the decrease in smooth muscle Ca^{2+} and dilation of the vessel. Thus, during EDHF-mediated dilations, the concentrations of Ca^{2+} in endothelial and smooth muscle cells change in opposite directions.

The evidence for a role of endothelial Ca^{2+} is based on the following observations. (1) Direct measurements in endothelial cells showed that relatively large increases in Ca^{2+} occurred with EDHF dilations.[77] (2) Ca^{2+} ionophores, which directly increase endothelial Ca^{2+} by selectively increasing the membrane conductance to Ca^{2+}, elicited EDHF-mediated dilations without involvement of receptor stimulation.[26,64,77–81] (3) Cyclopiazonic acid, which stimulates Ca influx *via* capacitative Ca entry, elicited an EDHF response.[82] (4) EDHF dilations are inhibited by preventing Ca^{2+} influx through nonselective cation channels.[83]

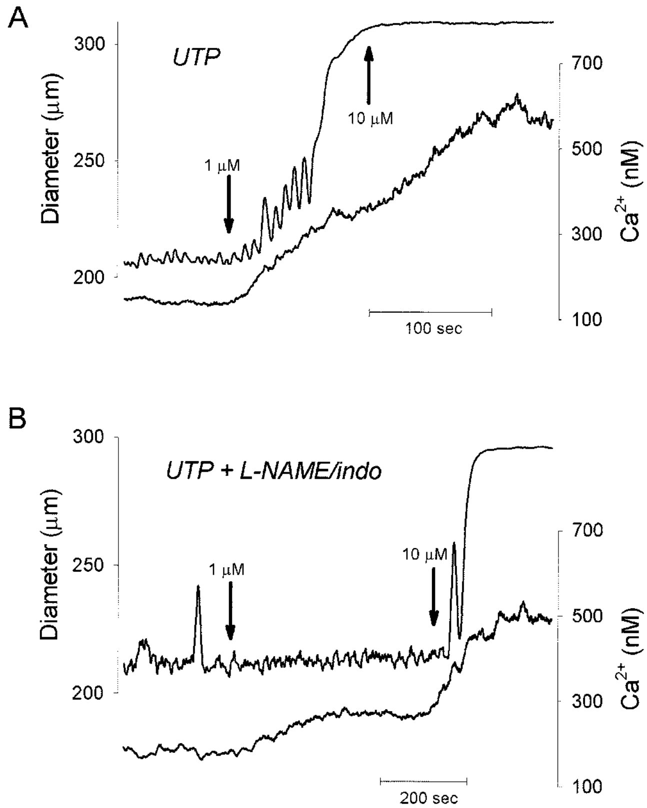

Fig. 3. Simultaneous measurement of middle cerebral artery diameter and endothelial Ca^{2+} with the addition of uridine triphosphate (UTP), an agonist for $P2Y_2$ receptors, in a control artery (*A*) and after inhibition of nitric oxide synthase and cyclooxygenase with N^G-nitro-L-arginine methylester (L-NAME) and indomethacin (indo) (*B*).

On stimulation of endothelial receptors, Ca^{2+} is thought to be initially released from internal stores through activation of phospholipase C and inositol trisphosphate–gated Ca^{2+}-release channels.[26,84–87] This initial Ca^{2+} increase is sustained by an influx of Ca^{2+} into the endothelial cell from the extracellular milieu.[82,83,86,87] In pressurized cerebral arteries, the resting Ca^{2+} concentration in endothelial cells ranges from 130 to 160 nM.[27,64,77,84,88,89] On stimulation of endothelial receptors with ATP or uridine triphosphate (agonists for $P2Y_2$ receptors), endothelial Ca^{2+} increased to 400–700 nM and elicited an EDHF dilation[27,64,77,84,90] (fig. 3). The Ca^{2+} threshold for eliciting an EDHF-mediated dilation is approximately 340 nM.[77] For comparison, the Ca^{2+} threshold for activation of NOS in the same artery is approximately 230 nM.[77]

The manner in which the increase in endothelial Ca^{2+} transitions into the next step of the EDHF mechanism is a controversial point. It is at this step where the proposed mechanisms for EDHF diverge. The major mechanisms currently being considered to explain EDHF dilations are (1) arachidonic acid metabolites, (2) the monovalent cation, K^+, (3) gap junctions, (4) and hydrogen peroxide. Other candidates for EDHF have been suggested, but evidence for these does not warrant discussion at this time.[91–94] The major proposed mechanisms of EDHF are discussed in greater detail below.

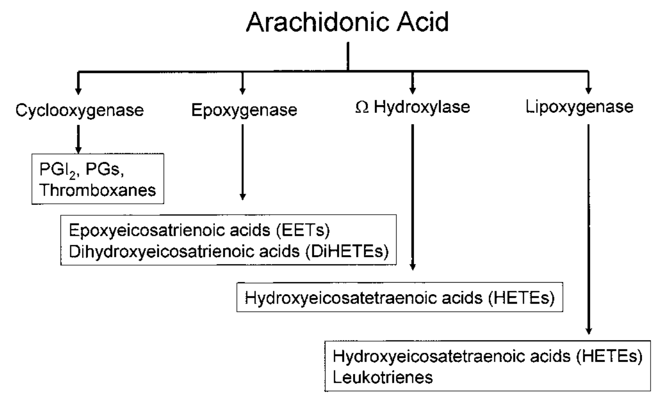

Fig. 4. Pathways of arachidonic acid metabolism. PGI_2 = prostacyclin, PGs = prostaglandins.

Mechanism 1: Arachidonic Acid Metabolites

Arachidonic Acid Metabolism through the Epoxygenase Pathway. During EDHF-mediated dilations, endothelial Ca^{2+} can increase to 400–700 nM.[27,64,77,84,90] At a Ca^{2+} concentration of 450 nM, 70% of the phospholipase A_2 (PLA_2) is translocated from the cytoplasm to cellular membranes.[95] PLA_2 is a lipase that hydrolyzes the linkage at the 2 position of the glycerophosphate backbone of membrane phospholipids. The major product of the hydrolysis is arachidonic acid. Because PLA_2 is constitutively active, the translocation to the membrane places it in contact with the phospholipid substrate and promotes the release of arachidonic acid within the cell. A role for PLA_2 involvement with the EDHF mechanism has been demonstrated by studies using pharmacologic inhibitors.[26,84,96,97]

The liberated arachidonic acid has several possible fates. It can be reincorporated into the membrane phospholipids; it can act as a messenger; or it can be metabolized further by COX, epoxygenase, lipoxygenase, or Ω hydroxylase (fig. 4).

The first mechanism proposed for EDHF dilations involves the metabolism of arachidonic acid through the epoxygenase pathway to form epoxyeicosatrienoic acids. In this model (fig. 5), activation of the endothelial receptor increases cytoplasmic free Ca^{2+}. The increase in Ca^{2+} in turn elicits the translocation of PLA_2 to the membrane and the subsequent liberation of arachidonic acid from the membrane phospholipids. Arachidonic acid is metabolized by epoxygenase, an enzyme with a cytochrome P-450 moiety, to EETs. The EETs diffuse from the endothelium to the vascular smooth muscle, where they activate a large conductance calcium-activated K channel (BK_{Ca}). Opening of the BK_{Ca} channel results in K^+ efflux from the smooth muscle cell, hyperpolarization, and dilation as described previously.

The idea that EETs are EDHF is based on several observations. (1) Selective inhibition of cytochrome P-450

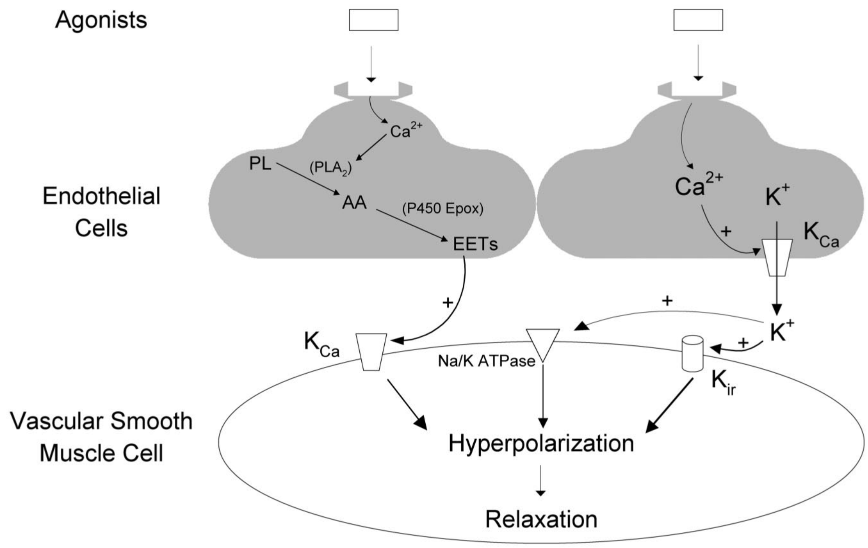

Fig. 5. Diagram of two putative mechanisms for endothelium-derived hyperpolarizing factor, epoxyeicosatrienoic acids (EETs) and K^+. AA = arachidonic acid; ATPase = adenosine triphosphatase; K_{Ca} = Ca-activated potassium channel; K_{ir} = inwardly rectifying potassium channel; P450 Epox = cytochrome P-450 epoxygenase; PL = membrane phospholipids; PLA_2 = phospholipase A_2.

epoxygenases by pharmacologic means and by antisense oligonucleotides blocked EDHF dilations.[98,98–102] (2) An antagonistic EETs analog blocked EDHF-mediated dilations.[103] (3) EETs are produced by endothelium.[101,102,104,105] (4) EETs and their metabolites dilate vessels by increasing the open state probability of BK_{Ca} channels and hyperpolarizing vascular smooth muscle.[102,105] (5) EDHF-mediated dilations are enhanced by agents that increase expression of cytochrome P-450 epoxygenase.[101,106] (6) Endothelium releases a transferable factor similar to P-450 epoxygenase products.[21,106,107]

A role for EETs as EDHF comes mostly from coronary and renal arteries,[36,100,102,108,109] although EDHF dilations in other vessels including skeletal muscle seem to involve a similar pathway.[98,100] In hepatic, cerebral, and mesenteric arteries, an involvement of this pathway could not be demonstrated using pharmacologic inhibitors of P-450 epoxygenase.[110–112]

However, these latter studies using P-450 epoxygenase inhibitors do not necessarily exclude a potential role for previously synthesized and stored EETs. EETs may be stored in the phospholipid pool in the 2 position of the glycerophosphate backbone, the same location as stored arachidonic acid.[113–117] EETs could be liberated by the direct action of PLA_2 without the immediate need for P-450 epoxygenase. Therefore, the P-450 epoxygenase inhibitors would not be effective in inhibiting EDHF-mediated dilations until all stored EETs had been depleted.[118]

If the EETs hypothesis (fig. 5) is valid, iberiotoxin, a specific inhibitor of BK_{Ca} (table 1), should block EDHF-mediated dilations. Iberiotoxin alone does inhibit EDHF dilations in coronary arteries[21,119] supporting the idea that EETs serve as an EDHF. However, iberiotoxin is not an effective inhibitor of EDHF in hepatic, cerebral, and mesenteric arteries.[110–112,120] Therefore, for these latter arteries, EETs do not seem to be an EDHF.

An alternative to the above idea involves EETs as key messengers, modulators, or amplifiers in the EDHF mechanism without being the actual EDHF, *i.e.*, a factor that diffuses from the endothelium to hyperpolarize vascular smooth muscle.[121] Metabolites of the cytochrome P-450 epoxygenase may regulate Ca^{2+} entry into endothelial cells,[82,122–124] activate K_{Ca} channels on endothelium,[125,126] and regulate gap junctions.[127] As discussed previously, regulation of Ca^{2+} in endothelium is a critical step in EDHF dilations. Activation of endothelial potassium channels and conduction through gap junctions are important steps in other models of EDHF (discussed below).

In summary, it is reasonable to consider that EDHF is a metabolite of arachidonic acid produced by the P-450 epoxygenase pathway in coronary, renal, and possibly skeletal muscle vascular beds. Alternatively, P-450 epoxygenase metabolites may serve as messengers or modulators in the EDHF pathway, but EETs *per se* do not seem to be the EDHF. However, it must be emphasized that species differences, conditions (physiologic, pathologic, or both), methods for studying the isolated vessels, and even diet could have major impacts on the EDHF mechanism and the involvement of EETs.[128]

Arachidonic Acid Metabolism through the Lipoxygenase Pathway. In addition to the epoxygenase pathway, metabolism of arachidonic acid through the lipoxygenase pathway may also be involved with EDHF dilations. Several metabolites of arachidonic acid through the lipoxygenase pathway dilate arteries through activation of potassium channels.[129–131] At least one of these metabolites seems to be associated with EDHF dilations to acetylcholine in the rabbit aorta.[132]

Mechanism 2: Potassium (K^+). Vascular smooth muscle contains many types of potassium channels. One type commonly found in the membranes of smooth muscle is the inwardly rectifying potassium channel (K_{ir};

table 1). K_{ir}s are responsive to increases in extracellular K^+. When extracellular K^+ increases from approximately 4 mM during rest to approximately 8 mM, K_{ir}s become activated.[133–137] Although the name of this potassium channel can be misleading, K^+ ions move in the same direction through the K_{ir} as with other potassium channels. Thus, under physiologic conditions, the electrochemical gradient favors K^+ movement out of the smooth muscle cell. The loss of positively charged K^+ results in hyperpolarization and subsequent dilation of the artery. At extracellular K^+ of 20–30 mM, the depolarizing effect of K^+ begins to offset any hyperpolarizing effects of K_{ir} activation.

A second method whereby extracellular K^+ can hyperpolarize vascular smooth muscle is by activation of Na/K adenosine triphosphatase (ATPase). At the expense of ATP, this enzyme exchanges three intracellular Na^+s for two extracellular K^+s. This net loss of a positive charge from the cell results in hyperpolarization. Na/K ATPase is activated by a number of mechanisms, one of which is an increase in extracellular K^+. Not all isoforms of Na/K ATPase can hyperpolarize vascular smooth muscle during physiologic conditions. One type of Na/K ATPase is fully activated at basal extracellular K^+ concentrations (approximately 4 mM). Therefore, any increase in extracellular K^+ could not further stimulate this isoform to hyperpolarize the vascular smooth muscle. However, two isoforms of Na/K ATPase that have a lower affinity for K^+ can be activated when K^+ increases above basal concentrations.[138,139] Dilations produced by increasing K^+ above basal concentrations require that the lower affinity Na/K ATPase isoforms are present.[139,140]

The model for K^+ as an EDHF is based on studies by Edwards *et al.*[141] (fig. 5). In this model, activation of endothelial receptors opens small and intermediate conductance calcium-activated potassium channels (SK_{Ca} and IK_{Ca}) on endothelium by increasing cytoplasmic Ca^{2+}. Intracellular K^+ moves down its electrochemical gradient through the open channels to the extracellular space. As a result of the ion movement, K^+ increases from approximately 4 mM to approximately 12 mM in the extracellular space located between endothelium and vascular smooth muscle. The increase in extracellular K^+ activates both the K_{ir} (table 1) and Na/K ATPase in the membrane of the vascular smooth muscle (fig. 5), resulting in hyperpolarization of the smooth muscle. Movement of K^+ to the extracellular space from the smooth muscle through the K_{ir} also helps to sustain increased extracellular K^+ concentrations. The hyperpolarization of the vascular smooth muscle by K_{ir} and Na/K ATPase elicits dilation as described previously.

One major difference between the EETs model and the K^+ model for EDHF involves the cellular location of K_{Ca} channels. The K_{Ca}s in the EETs model are located on the vascular smooth muscle, whereas the K_{Ca}s are located on the endothelium for the K^+ model. Several studies have demonstrated that the IK_{Ca} and SK_{Ca} involved with EDHF dilations are located on the endothelium and that the hyperpolarization of the endothelium by activation of these channels is necessary for agonist-induced EDHF dilations.[27,141–145] Although the hyperpolarization of the endothelium by IK_{Ca} and SK_{Ca} is consistent with the K^+ model, it is also consistent with models involving gap junctions (see "Mechanism 3: Gap Junctions") and is not necessarily inconsistent with the EETs model.

Inhibition of both K_{ir} and Na/K ATPase with Ba^{2+} (table 1) and ouabain, respectively, blocked EDHF dilations in rat hepatic arteries in addition to blocking the related hyperpolarization of smooth muscle.[141] Similarly, the same combination of Ba^{2+} and ouabain blocked K^+-induced hyperpolarizations and dilations when extracellular K^+ was increased from 5 to 10 mM. Thus, K^+ mimicked EDHF.

K^+, measured in or near the myoendothelial space, increased from approximately 5 mM to 11 mM on the addition of acetylcholine.[141] A combination of charybdotoxin and apamin (table 1) blocked the hyperpolarization of the endothelium produced by acetylcholine and blocked the increase in K^+ in the myoendothelial space.[141] The above data reported by Edwards *et al.*[141] and a number of subsequent studies provide evidence in support of the model shown in figure 5.

Other studies have disputed the finding that K^+ serves as an EDHF.[45,146–152] The conclusion of the above studies was based on the inability of Ba^{2+} and ouabain to inhibit EDHF dilations and the fact that increasing extracellular K^+ did not mimic EDHF dilations.

In summary, there is good evidence in the literature supporting the idea that K^+ is an EDHF in some arteries. However, there are other studies that oppose the K^+ hypothesis. It must be noted that all of the above studies were conducted in *ex vivo* vessels. Seemingly subtle differences in experimental conditions could alter the mechanism of the dilation and thus account for the differences between investigators.[153,154] Further studies are required to determine the role of K^+ as an EDHF *in vivo*.

Mechanism 3: Gap Junctions. Gap junctions are intercellular channels that allow passage of small water-soluble molecules (< 1,000 Da) including cyclic adenosine monophosphate, cyclic guanosine monophosphate, inositol triphosphates, and inorganic ions but do not allow proteins to pass from cell to cell. There is some selectivity for cations over anions.[16,155,156] Gap junctions consist of connexins, which are protein subunits with four transmembrane-spanning domains (fig. 6A). Six connexin subunits are required to form a connexon or hemichannel. Two adjacent cells each provide a hemichannel, and the hemichannels dock to form a complete gap junction. More than a dozen connexins have been identified, of which connexins 37, 40, 43, and 45 have been identified in vessels.[157,158] Gap junctions

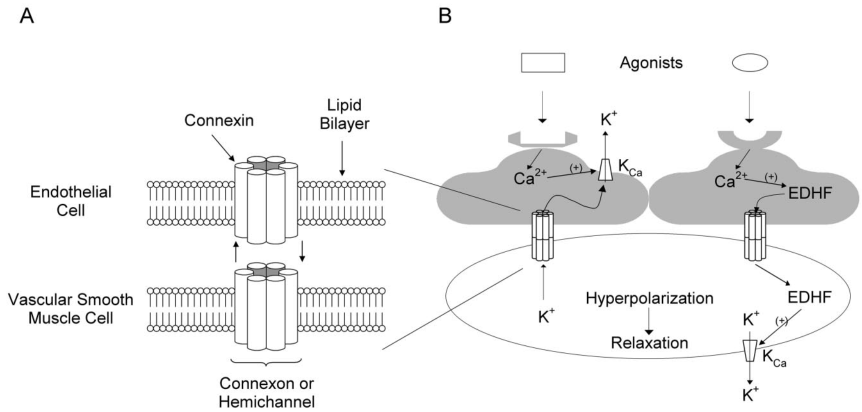

Fig. 6. (*A*) Diagram of a gap junction between an endothelial and a vascular smooth muscle cell. (*B*) Mechanisms of gap junction involvement in endothelium-derived hyperpolarizing factor (EDHF) dilations. K_{Ca} = Ca-activated potassium channel.

can be regulated by Ca^{2+}, voltage, pH, phosphorylation, ATP, or EETs.[127,159–162]

Although it has been known that gap junctions exist between endothelial cells and between vascular smooth muscle cells, they also seem to exist between endothelium and vascular smooth muscle (myoendothelial gap junctions).[163] Functional or indirect evidence, including electrical conductivity and transfer of dye between adjacent cells, supports the existence of myoendothelial gap junctions.[158,164,165] These myoendothelial gap junctions are thought to have a major role in the EDHF response by relaying the dilator signal from the endothelium to the vascular smooth muscle.

The study of gap junctions has been hampered in the past by a lack of specific inhibitors.[16,166,167] Recently, peptide inhibitors, which seem to have greater selectivity for gap junctions, have been used in the study of EDHF.[167] The peptide inhibitors, termed *gap peptides*, consist of amino acid sequences identical to portions of extracellular loops of the connexin proteins. It is thought that these gap peptides interfere with docking of hemichannels between adjacent cells. One gap peptide (11 amino acids), based on the extracellular loop of connexins 37 and 43, blocked dye transfer in cultured cells.[168,169] The same gap peptide inhibited EDHF-mediated dilations in isolated superior mesenteric artery and aorta of the rabbit and cerebral pial arterioles *in vivo*.[66,167] In the cerebral pial arterioles, antisense oligonucleotides directed against connexin 43 blocked the EDHF-like dilation.[66] The antisense study amplified the conclusion involving gap junctions and confirmed the efficacy of the gap peptide. The connexin composition of the gap junctions involved with EDHF dilations varies among arteries. Connexin 37, 40, and 43 have been identified as the protein building blocks of gap junctions important in EDHF dilations.[66,167,168,170] Therefore, it seems that there is heterogeneity of gap junction types involved with the EDHF mechanism in different arteries.

Sandow *et al.*[171] conducted an elegant study comparing arteries that did and did not have an EDHF-mediated dilation. The rat mesenteric artery, which has EDHF-mediated dilations, contains anatomically identified myoendothelial gap junctions and tight electrical coupling between endothelial and vascular smooth muscle cells. On the other hand, the rat femoral artery, which does not have an EDHF dilation, contained no myoendothelial gap junctions and showed no electrical coupling between endothelial and smooth muscle cells. The data from the different arteries provides further evidence for a role of myoendothelial gap junctions in EDHF dilations.

The gap peptides have served as major tools in studies where a role for myoendothelial gap junctions in EDHF dilations has been implicated. However, the gap peptides have two limitations. First, evidence is beginning to emerge that unpaired connexons or hemichannels can function as cellular pores, in addition to acting as half of a gap junction. The gap peptides used for blocking gap junctions also seem to block the function of a hemichannel pore.[172] Therefore, if a single hemichannel is a functional pore and can be inhibited by the gap peptides, it is possible that the hemichannel, not the gap junction, is the structure involved with EDHF dilations. Further studies are required to determine the role of hemichannels in cellular regulation and EDHF-mediated dilations. Second, the gap peptides do not selectively block only those gap junctions between endothelium and vascular smooth muscle. The inhibitors also block the gap junctions between endothelial cells and those between smooth muscle cells. Without selectivity of the peptide, an absolute requirement for myoendothelial gap junctions is questioned.[12,173]

Although there is evidence for gap junction involvement in many vessel types, a question remains as to what is conducted through the gap junctions for the endothelium to pass the appropriate signal to the smooth muscle. One possibility is that the gap junctions conduct the EDHF from endothelium to smooth muscle (fig. 6B). Another possible role for gap junctions is not the passage of EDHF *per se* but the passage of electrical current in the form of ions. Electrophysiologic studies in arteries from guinea pigs, rats, and humans demonstrated that EDHF involves electrical spread of hyperpolarization from the endothelial cells to the smooth muscle cells.[45,174] One possibility is that K^+ carries the current as shown in figure 6B. That is, potassium movement from the vascular smooth muscle to the endothelium *via* gap junctions would result in smooth muscle hyperpolarization. Figure 6B shows two possible scenarios where myoendothelial gap junctions could be involved with EDHF dilations. Movement of K^+ out of the vascular smooth muscle through gap junctions to the endothelium and ultimately to the extracellular space would produce a net hyperpolarization of the vascular smooth muscle. The second scenario would be for the EDHF to move from the endothelium to the vascular smooth muscle by way of the myoendothelial gap junctions.

Mechanism 4: Hydrogen Peroxide. Hydrogen peroxide (H_2O_2) dilates a number of arteries and arterioles by hyperpolarizing the vascular smooth muscle through activation of K_{Ca} or sometimes K_{ATP}.[19,47,175–183] H_2O_2 has been reported to be an EDHF in a number of arteries.[19,47,175–177] One hypothesis is that superoxide is generated on activation of endothelial nitric oxide. The superoxide is converted to H_2O_2 by the actions of superoxide dismutase.[176] The newly generated H_2O_2 diffuses to the vascular smooth muscle, where it activates K_{Ca} or K_{ATP}, hyperpolarizes the vascular smooth muscle, and elicits dilation.[19,175]

Hydrogen peroxide can also be produced by the action of superoxide dismutase on superoxide generated from COX, lipoxygenase, epoxygenase, xanthine oxidase, NADPH oxidase, and sites along the mitochondrial respiratory chain.[19,176,184,185]

The idea that H_2O_2 is an EDHF is based on the facts that (1) catalase, an enzyme that catalyzes the decomposition of H_2O_2 to H_2O and O_2, attenuates EDHF dilations; (2) H_2O_2 and EDHF dilate by a similar mechanism; and (3) H_2O_2 production is increased with the EDHF response.[19,175–177,185]

However, just as there is evidence for H_2O_2 in the mechanism of EHDF, there is also evidence against H_2O_2. A number of studies have not found catalase to effectively inhibit EHDF dilations.[46,186–188] Furthermore, dilations elicited by H_2O_2 do not always mimic EDHF dilations.[189]

Pathologic Significance

After many pathologic conditions, dilation produced by endothelium-derived nitric oxide can be significantly attenuated. The primary reasons for the attenuated dilations include an excessive production of reactive oxygen species, which inactivate nitric oxide, and/or dysfunction in eNOS generation of nitric oxide.[190–195] In contrast, EDHF seems to be resistant to reactive oxygen species.[196] In fact, EDHF has been reported to be upregulated after a variety of pathologic conditions when nitric oxide-mediated dilations have been attenuated. The up-regulation seems to occur after ischemia–reperfusion, traumatic injury, congestive heart failure, coronary artery disease, hypercholesterolemia, and angioplasty.[13,19,81,90,197–202] Of note, patients with congestive heart failure showed an up-regulated EDHF-like dilation in the forearm circulation after administration of acetylcholine.[58,203] In a rat model of hyperthyroidism, EDHF was up-regulated 36 h after triiodothyronine treatment in renal arteries, but it was down-regulated after 8 weeks.[204] Figure 7 shows dilations of branches of middle cerebral arteries taken from rats 1 day after a mild head injury or after sham injury.[197] The dilations were elicited by the P2Y$_2$ receptor agonist ATP. In sham-injured rats, inhibition of NOS (N^G-nitro-L-arginine methylester) and COX (indomethacin) shifted the response to ATP 10-fold to the right (fig. 7A). The dilation after NOS and COX was mediated by EDHF.[24,197] In arteries from injured rats, inhibition of NOS and COX had no effect on the dilation (fig. 7B). Figure 7C shows the up-regulation of the EDHF response by a direct comparison in the two groups.

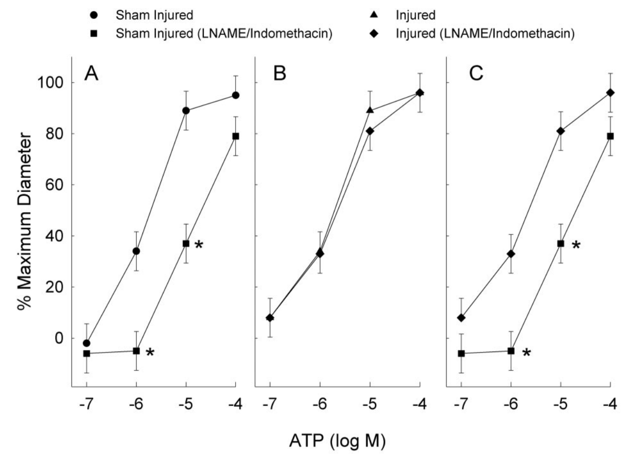

Fig. 7. Dilations in third-order branches of the rat middle cerebral artery from control (sham-injured) (*A*) and head-injured rats (*B*). (*C*) A direct comparison of endothelium-derived hyperpolarizing factor dilations from branches of the rat middle cerebral artery from control and head-injured rats. A controlled cortical impact model was used for the injury.[197] Endothelium-dependent dilations were elicited by adenosine triphosphate (ATP), an agonist for P2Y$_2$ receptors. N^G-nitro-L-arginine methylester (L-NAME) and indomethacin are inhibitors of nitric oxide synthase and cyclooxygenase, respectively.

During hypertension, EDHF has been reported to be either enhanced or suppressed.[205–211] Pulmonary hypertension in sheep enhanced EDHF in the pulmonary artery.[212] Other conditions where EDHF has been reported to be suppressed include aging[29,205,210,213] and type I diabetes.[214–219] EDHF has been reported to be either enhanced or suppressed in animal models of type II diabetes.[220,221]

The effect of the pathologic condition on the EDHF response could be a result of multiple factors. In some pathologic conditions, the metabolic pathways that regulate EDHF could be compromised (producing down-regulation), whereas in other cases, up-regulation of EDHF could be a response to the pathologic conditions.[222] In addition, the effect of the pathologic condi-

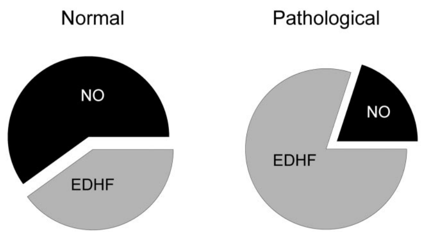

Fig. 8. Relative contributions of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) to the overall dilations during normal and pathologic conditions. When the nitric oxide component of a dilation is attenuated during pathologic conditions, EDHF can be up-regulated to maintain a near-normal dilation.

tion on the EDHF dilation could be related to the vessel size, the vascular bed being studied, or the severity and duration of the pathologic condition.

Although EDHF dilations are up-regulated in both large and small arteries in certain pathologic conditions, there seems to be a greater propensity for up-regulation in smaller vessels. EDHF responses in eNOS null mice support this idea. Larger conduit arteries typically showed no up-regulation of EDHF in eNOS null mice, whereas up-regulation of EDHF did occur in smaller "resistance-sized" arteries and arterioles.[223–228]

In those pathologic conditions where EDHF is up-regulated, it is thought to be a protective mechanism that compensates for insufficient endothelium-derived nitric oxide. A number of studies suggest that there is a balance in the nitric oxide and EDHF response.[197,199,200,229–231] When the nitric oxide–mediated dilation is impaired during pathologic conditions, EDHF is up-regulated sufficiently to maintain near-normal dilation (fig. 8). Thus, the relative contributions of nitric oxide and EDHF to the overall dilation are adjusted accordingly for the response to remain relatively unchanged. An example during traumatic brain injury is shown in figure 7.

In disease states, activated eNOS can generate superoxide anions after depletion of tetrahydrobiopterin or L-arginine.[19,176,190] In the presence of superoxide dismutase, the superoxide anions are converted to H_2O_2, a putative EDHF (see "Mechanism 4: Hydrogen Peroxide"). This scenario represents one of many ways that the contribution of endothelium-derived H_2O_2 could be increased in disease states.[19,190]

Interaction with Anesthetics

There is good agreement in the literature that anesthetics in general suppress EDHF dilations (tables 2 and 3). For the inhalation anesthetics, isoflurane and sevoflurane are more potent inhibitors than desflurane, enflurane, or halothane on a molar basis.[232] Ketamine, propofol, and all barbiturates, with the exception of phenobarbital, inhibited the EDHF dilations (tables 2 and 3). Etomidate (1 μM) enhanced EDHF dilations in arteries but suppressed it at greater concentrations.[39,48,233] Further evidence showed that isoflurane (1.4%) suppressed the EDHF dilation when compared with either halothane (1.2%) or ketamine in rat cremaster arterioles (150 mg/kg followed by an infusion of 1.5 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}).[53]

Lischke *et al.*[232,234] demonstrated that all volatile anesthetics studied, etomidate, thiopental, and methohexital, but not phenobarbital, inhibited cytochrome P-450 activity in rabbit liver microsomes. The authors suggested that anesthetics inhibit the cytochrome P-450 epoxygenase, the enzyme family responsible for metabolizing arachidonic acid to EETs.[232,234] However, as the authors pointed out, the cytochrome P-450 in the liver microsomes is likely different from the cytochrome P-450 epoxygenase that synthesizes EETs.[234]

Anesthetics in general seem to inhibit EDHF dilations. One possible mechanism for blocking EDHF could be through inhibition of cytochrome P-450 epoxygenase, a putative "EDHF synthase." Although inhalation anesthetics could block EDHF dilations by their ability to block gap junction communication,[235,236] the concentration required to block gap junctions is above clinically relevant halothane concentrations.[232,237] In arteries where P-450 epoxygenase does not seem to be involved with EDHF dilations, a mechanism of inhibition by anesthetics is lacking. Although anesthetics seem to inhibit EDHF dilations, they do not abolish them. Therefore, there is

Table 2. Effects of Volatile Anesthetics on EDHF Dilations

Anesthetic	Concentration, %	MAC	Vessel or Tissue	EDHF Dilation	Reference
Halothane	2	1.4% for rabbit[259] 1% for rat[260]	Rabbit carotid artery	36% inhibition	Lischke *et al.*,[232] 1995
	1–3		Rat mesenteric artery	54–86% inhibition	Iranami *et al.*,[261] 1997
Isoflurane	2	2% for rabbit[259]	Rabbit carotid artery	32% inhibition	Lischke *et al.*,[232] 1995
	2		Rabbit small mesenteric artery	55% inhibition	Akata *et al.*,[262] 1995
	2	1.1% for rat[260]	Isolated perfused rat heart	61% inhibition	Lischke *et al.*,[233] 1995
Sevoflurane	2	3.7% for rabbit[263]	Rabbit carotid artery	27% inhibition	Lischke *et al.*,[232] 1995
	3.7		Rabbit small mesenteric artery	27% inhibition	Akata *et al.*,[262] 1995
Desflurane	8	8.9% for rabbit[264]	Rabbit carotid artery	34% inhibition	Lischke *et al.*,[232] 1995
Enflurane	2	2.8% for rabbit[259]	Rabbit carotid artery	24% inhibition	Lischke *et al.*,[232] 1995
	2.8		Rabbit small mesenteric artery	37% inhibition	Akata *et al.*,[262] 1995

EDHF = endothelium-derived hyperpolarizing factor.

Table 3. Effects of Intravenous Anesthetics on EDHF Dilations

Anesthetics	Concentration	Vessel or Tissue	EDHF Dilation	Reference
Barbituate anesthetics				
Methohexital	0.03 and 0.3 mM	Rabbit carotid artery	24 and 37% inhibition	Lischke *et al.*,[234] 1995
Pentobarbital	75 mg/kg intraperitoneally	Hamster skin muscle arterioles *in vivo*	64% inhibition	De Wit *et al.*,[265] 1999
	1 and 2 mM	Hamster femoral artery	34 and 50% inhibition	De Wit *et al.*,[265] 1999
Phenobarbital	0.1 and 0.3 mM	Rabbit carotid artery	No effect	Lischke *et al.*,[234] 1995
	0.1 mM	Isolated perfused rat heart	No effect	Lischke *et al.*,[233] 1995
Thiopental	0.3 mM	Human renal artery	38% inhibition	Kessler *et al.*,[39] 1996
	0.03 and 0.1 mM	Isolated perfused rat heart	32 and 43% inhibition	Lischke *et al.*,[233] 1995
	0.1 and 0.3 mM	Rabbit carotid artery	60 and 87% inhibition	Lischke *et al.*,[234] 1995
Other intravenous anesthetics				
Etomidate	0.3 mM	Human renal artery	38% inhibition	Kessler *et al.*,[39] 1996
	0.03 and 0.1 mM	Isolated perfused rat heart	33 and 63% inhibition	Lischke *et al.*,[233] 1995
	10^{-6} M	Human omental artery	26% enhancement Increased EC_{50} 2.4-fold	Bodelsson *et al.*,[48] 2000
	10^{-4} M	Human omental artery		
Propofol	3 μM to 0.1 mM	Isolated canine pulmonary artery	Increased EC_{50} 3-fold 9% inhibition of maximum dilation	Horibe *et al.*,[266] 2000

EC_{50} = effective concentration for one half of the maximal response; EDHF = endothelium-derived hyperpolarizing factor.

the potential to augment or further inhibit EDHF dilations as necessary in the operating room (see "Clinical Implications"). More studies are needed to determine how anesthetics affect EDHF dilations and what this inhibition would mean to the clinical practice of anesthesiology.

Clinical Implications

Anesthesiologists and intensivists have to deal with two problems involving the cardiovascular system. First, blood pressure must to be maintained for perfusion of vascular beds. Second, anesthesiologists and intensivists must ensure that adequate blood flow to vital organs is maintained. Compared with other organs of the body, the brain, heart, and kidney are relatively more sensitive to interruptions in the blood supply. Although the study of EDHF is relatively new and is not currently a clinical consideration, manipulating it does have potential for controlling both blood pressure and maintaining blood flow to vital organs. As the future clinical potential is considered for EDHF, parallels to its cousin, nitric oxide, will provide some insight.

The nitric oxide–cyclic guanosine monophosphate system is used clinically to control blood pressure and to maintain blood perfusion to selected tissues, including heart, brain, and lung.[238–249] For example, inhaled nitric oxide is effective in reversing conditions affecting the pulmonary vasculature, including persistent pulmonary hypertension of newborns, hypoxia-induced pulmonary hypertension, and adult respiratory distress.[239,250] In addition, nitric oxide has also been used in the treatment of cerebral vasospasm by selectively applying nitric oxide donors to the vasospastic artery.[240]

The potential does exist for EDHF to be used in similar ways as nitric oxide to control blood pressure and to maintain blood perfusion to vital organs. In fact, manipulations of EDHF in conjunction with nitric oxide could prove to be more effective during conditions where nitric oxide therapy alone has been met with limited success.[250,251]

For controlling blood pressure, EDHF could be manipulated to increase or decrease the pressure as required. Enhancement of EDHF to globally dilate vessels would act to decrease blood pressure. Conversely, global inhibition of EDHF would constrict vessels and increase blood pressure. Major contributors to the maintenance of blood pressure are the small arteries and arterioles (< 300 μm).[252] Importantly, it is these vessels that seem to have a more pronounced EDHF response, possibly at the expense of endothelium-derived nitric oxide.[24,28–32] Manipulation of the EDHF system could therefore provide as great or even greater control of blood pressure than manipulation of the nitric oxide–cyclic guanosine monophosphate system. For example, overexpression in mice of SK3, the small conductance calcium-activated K channel involved with EDHF, hyperpolarized both the endothelium and vascular smooth muscle, dilated arteries *in vivo* and *in vitro*, and decreased blood pressure.[253] Conversely, decreasing the expression of the SK3 had the opposite effects: vessel constriction and increased blood pressure.[253] Manipulation of EDHF could therefore be an important means to regulate systemic blood pressure. It follows that a better understanding of the mechanisms controlling EDHF is a necessary step to reach the important endpoint of manipulating EDHF therapeutically.[23]

Manipulation of EDHF could be used to maintain blood flow to vital organs after compromise of the vascular system. There are two considerations regarding EDHF that are relevant. First, as stated previously, the smaller resistance-sized arteries and arterioles have a more pronounced EDHF response.[24,28–32] It is these smaller vessels where the major resistance to blood flow occurs and is thus the major control point of blood flow. Manipulation of these smaller sized vessels through EDHF could be an effective and efficient means to control blood flow in a vascular bed. Second, the mechanism for EDHF seems to be different in different vascular beds. This fact could be exploited to stimulate EDHF, vessel dilation, and blood flow to a selective organ without affecting resistance in the vascular beds of other organs. For example, pharmacologic agents could possibly stimulate the EDHF pathway in cerebral arteries and arterioles to selectively reduce the cerebrovascular resistance without altering the resistance in other vascular beds. Because global resistance would be minimally affected, cerebral perfusion would increase with no or very little change in blood pressure. Another example would be to selectively activate EDHF in the kidney to increase blood flow and restore urine output in a patient whose renal blood flow has been decreased to critical rates. Again, the selectivity of the dilation compared with a global dilation would ensure that kidney vascular resistance would be decreased without affecting the overall vascular resistance.

Interestingly, the anesthesiologist may already be manipulating EDHF during cardiac bypass when using pulsatile flow. Pulsatile flow decreases vascular resistance by enhancing the release of nitric oxide through mechanical deformation of endothelial cells.[254–257] Pulsatile flow also elicits EDHF dilations.[21,258] Perhaps enhanced EDHF dilations work in conjunction with endothelium-derived nitric oxide to improve tissue perfusion with pulsatile flow. During hypoxia, when dilations by endothelium-dependent nitric oxide are inhibited, EDHF, which is not affected by hypoxia,[22] may contribute more to the decreased vascular resistance than nitric oxide during bypass with pulsatile flow.

Often, it is very sick patients who come to the operating room or intensive care unit. As stated earlier, pathologic conditions often affect EDHF to either suppress or enhance the response. With knowledge of the mechanism of EDHF and how the EDHF response is affected by a particular condition, EDHF could be exploited in a beneficial way to provide a combination of desirable pressure maintenance and adequate flow to vital organs in individual disease states.

Summary

There is overwhelming evidence that an endothelial mechanism, other than nitric oxide or prostacyclin, exists for dilating arteries and arterioles. The third pathway is characterized by hyperpolarization of the vascular smooth muscle and involvement of potassium channels, most often small and intermediate conductance calcium-activated potassium channels (IK_{Ca} and SK_{Ca}). EDHF is more prevalent in smaller resistance-sized arteries and arterioles than in larger conduit arteries. Because these resistance-sized vessels are more significant in the regulation of blood flow, EDHF may have a major but relatively unrecognized role in the control of flow during normal physiologic conditions. During some pathologic states, EDHF can be up-regulated. This up-regulation often occurs as the dilator effects of endothelium-derived nitric oxide are suppressed. The up-regulated EDHF may serve in a protective capacity to help maintain blood flow to organs and tissues during these stressful states.

The most controversial aspect of EDHF research is the mechanism. Arachidonic acid metabolism to EETs through the epoxygenase pathway, K^+, gap junctions, and H_2O_2 are the most widely studied mechanisms, although others do exist. There is evidence for and against each of the above mechanisms. These principal mechanisms are not necessarily mutually exclusive and could possibly coexist. However, there are likely multiple mechanisms for EDHF. In an individual vessel, the mechanism for EDHF likely depends on the species, organ, vessels size, diet, hormonal state, environmental conditions, and presence or absence of a pathologic condition. As a case in point, the P-450 epoxygenase pathway can be readily altered as a result of the above conditions.[128]

The discovery of a new endothelial-mediated dilatory process is intriguing, but many questions must be answered before the true therapeutic potential of EDHF can be fully recognized. Currently, EDHF is almost exclusively studied after inhibition of both nitric oxide and the COX pathway. Only when the mechanism of EDHF-mediated dilations is elucidated can it be studied in the presence of nitric oxide and COX metabolites, and only then can its relative importance in the control of blood flow during normal physiologic conditions be determined. We as scientists are challenged to understand the mechanism, role, and clinical implications of EDHF.

References

1. Furchgott RF, Vanhoutte PM: Endothelium-derived relaxing and contracting factors. FASEB J 1989; 3:2007–18
2. Boulanger CM, Vanhoutte PM: The Endothelium: A Pivotal Role in Health and Cardiovascular Disease. Courbevoie, France, Servier International, 1994, pp 11–20
3. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288:373–6
4. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G: Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci U S A 1987; 84:9265–9
5. Palmer RM, Ferrige AG, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987; 327: 524–6
6. Bolotina VM, Najibi S, Palacino JJ, Pagano PJ, Cohen RA: Nitric oxide

directly activates calcium-dependent potassium channels in vascular smooth muscle. Nature 1994; 368:850–3

7. Sun CW, Falck JR, Okamoto H, Harder DR, Roman RJ: Role of cGMP versus 20-HETE in the vasodilator response to nitric oxide in rat cerebral arteries. Am J Physiol Heart Circ Physiol 2000; 279:H339–50

8. Taylor SG, Weston AH: Endothelium-derived hyperpolarizing factor: A new endogenous inhibitor from the vascular endothelium. Trends Pharmacol Sci 1988; 9:272–4

9. Chen G, Suzuki H, Weston AH: Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. Br J Pharmacol 1988; 95:1165–74

10. Feletou M, Vanhoutte PM: Endothelium-dependent hyperpolarization of canine coronary smooth muscle. Br J Pharmacol 1988; 93:515–24

11. Golding EM, Marrelli SP, You J, Bryan RM Jr: Endothelium-derived hyperpolarizing factor in the brain: A new regulator of cerebral blood flow? Stroke 2002; 33:661–3

12. Edwards G, Weston AH: EDHF: Are there gaps in the pathway? (perspective). J Physiol 2001; 531:299

13. McLean PG, Aston D, Sarkar D, Ahluwalia A: Protease-activated receptor-2 activation causes EDHF-like coronary vasodilation: Selective preservation in ischemia/reperfusion injury: Involvement of lipoxygenase products, VR1 receptors, and C-fibers. Circ Res 2002; 90:465–72

14. Hamilton JR, Cocks TM: Heterogeneous mechanisms of endothelium-dependent relaxation for thrombin and peptide activators of protease-activated receptor-1 in porcine isolated coronary artery. Br J Pharmacol 2000; 130:181–8

15. Garland CJ, Plane F, Kemp BK, Cocks TM: Endothelium-dependent hyperpolarization: A role in the control of vascular tone. Trends Pharmacol Sci 1995; 16:23–30

16. McGuire JJ, Ding H, Triggle CR: Endothelium-derived relaxing factors: A focus on endothelium-derived hyperpolarizing factor(s). Can J Physiol Pharmacol 2001; 79:443–70

17. McGuire JJ, Hollenberg MD, Andrade-Gordon P, Triggle CR: Multiple mechanisms of vascular smooth muscle relaxation by the activation of proteinase-activated receptor 2 in mouse mesenteric arterioles. Br J Pharmacol 2002; 135:155–69

18. Huang A, Wu Y, Sun D, Koller A, Kaley G: Effect of estrogen on flow-induced dilation in NO deficiency: Role of prostaglandins and EDHF. J Appl Physiol 2001; 91:2561–6

19. Miura H, Bosnjak JJ, Ning G, Saito T, Miura M, Gutterman DD: Role for hydrogen peroxide in flow-induced dilation of human coronary arterioles. Circ Res 2003; 92:e31-40

20. Takamura Y, Shimokawa H, Zhao H, Igarashi H, Egashira K, Takeshita A: Important role of endothelium-derived hyperpolarizing factor in shear stress–induced endothelium-dependent relaxations in the rat mesenteric artery. J Cardiovasc Pharmacol 1999; 34:381–7

21. Popp R, Fleming I, Busse R: Pulsatile stretch in coronary arteries elicits release of endothelium-derived hyperpolarizing factor: A modulator of arterial compliance. Circ Res 1998; 82:696–703

22. Feletou M, Vanhoutte PM: EDHF: New therapeutic targets? Pharmacol Res 2004; 49:565–80

23. Ding H, Triggle CR: Relaxing blood vessels: Are there novel endothelium-derived mediators to be found and can their discovery lead to the development of new therapeutic agents? Pharmaceutical News 2001; 8:42–9

24. You J, Johnson TD, Marrelli SP, Bryan RM Jr: Functional heterogeneity of endothelial P2 purinoceptors in the cerebrovascular tree of the rat. Am J Physiol 1999; 277:H893–900

25. You JP, Johnson TD, Marrelli SP, Mombouli JV, Bryan RM Jr: P2u-receptor mediated release of endothelium-derived relaxing factor/nitric oxide and endothelium-derived hyperpolarizing factor from cerebrovascular endothelium in rats. Stroke 1999; 30:1125–33

26. Hutcheson IR, Chaytor AT, Evans WH, Griffith TM: Nitric oxide-independent relaxations to acetylcholine and A23187 involve different routes of heterocellular communication: Role of Gap junctions and phospholipase A2. Circ Res 1999; 84:53–63

27. Marrelli SP, Eckmann MS, Hunte MS: Role of endothelial intermediate conductance KCa channels in cerebral EDHF-mediated dilations. Am J Physiol Heart Circ Physiol 2003; 285:H1590–9

28. Tomioka H, Hattori Y, Fukao M, Sato A, Liu M, Sakuma I, Kitabatake A, Kanno M: Relaxation in different-sized rat blood vessels mediated by endothelium-derived hyperpolarizing factor: Importance of processes mediating precontractions. J Vasc Res 1999; 36:311–20

29. Urakami-Harasawa L, Shimokawa H, Nakashima M, Egashira K, Takeshita A: Importance of endothelium-derived hyperpolarizing factor in human arteries. J Clin Invest 1997; 100:2793–9

30. Shimokawa H, Yasutake H, Fujii K, Owada MK, Nakaike R, Fukumoto Y, Takayanagi T, Nagao T, Egashira K, Fujishima M, Takeshita A: The importance of the hyperpolarizing mechanism increases as the vessel size decreases in endothelium-dependent relaxations in rat mesenteric circulation. J Cardiovasc Pharmacol 1996; 28:703–11

31. Garland CJ, Plane F: Relative importance of endothelium-derived hyperpolarizing factor for the relaxation of vascular smooth muscle in different arterial beds, Endothelium-Derived Hyperpolarizing Factor. Edited by Vanhoutte PM. Amsterdam, Harwood, 1996, pp 173–9

32. Berman RS, Martin PE, Evans WH, Griffith TM: Relative contributions of NO and gap junctional communication to endothelium-dependent relaxations of rabbit resistance arteries vary with vessel size. Microvasc Res 2002; 63:115–28

33. Segal SS: Communication among endothelial and smooth muscle cells coordinates blood flow control during exercise. News in Physiological Sciences 1992; 7:152–6

34. Hoepfl B, Rodenwaldt B, Pohl U, De Wit C: EDHF, but not NO or prostaglandins, is critical to evoke a conducted dilation upon ACh in hamster arterioles. Am J Physiol Heart Circ Physiol 2002; 283:H996–1004

35. Welsh DG, Segal SS: Role of EDHF in conduction of vasodilation along hamster cheek pouch arterioles in vivo. Am J Physiol Heart Circ Physiol 2000; 278:H1832–9

36. Miura H, Liu Y, Gutterman DD: Human coronary arteriolar dilation to bradykinin depends on membrane hyperpolarization: Contribution of nitric oxide and Ca2+-activated K+ channels. Circulation 1999; 99:3132–8

37. Kemp BK, Cocks TM: Evidence that mechanisms dependent and independent of nitric oxide mediate endothelium-dependent relaxation to bradykinin in human small resistance-like coronary arteries. Br J Pharmacol 1997; 120:757–62

38. Petersson J, Zygmunt PM, Brandt L, Högestätt ED: Substance P-induced relaxation and hyperpolarization in human cerebral arteries. Br J Pharmacol 1995; 115:889–94

39. Kessler P, Lischke V, Hecker M: Etomidate and thiopental inhibit the release of endothelium-derived hyperpolarizing factor in the human renal artery. Anesthesiology 1996; 84:1485–8

40. Bussemaker E, Popp R, Binder J, Busse R, Fleming I: Characterization of the endothelium-derived hyperpolarizing factor (EDHF) response in the human interlobar artery. Kidney Int 2003; 63:1749–55

41. Angulo J, Cuevas P, Fernandez A, Gabancho S, Videla S, Saenz dT, I: Calcium dobesilate potentiates endothelium-derived hyperpolarizing factor-mediated relaxation of human penile resistance arteries. Br J Pharmacol 2003; 139:854–62

42. Archer SL, Gragasin FS, Wu X, Wang S, McMurtry S, Kim DH, Platonov M, Koshal A, Hashimoto K, Campbell WB, Falck JR, Michelakis ED: Endothelium-derived hyperpolarizing factor in human internal mammary artery is 11,12-epoxyeicosatrienoic acid and causes relaxation by activating smooth muscle BK(Ca) channels. Circulation 2003; 107:769–76

43. Wihlborg AK, Malmsjo M, Eyjolfsson A, Gustafsson R, Jacobson K, Erlinge D: Extracellular nucleotides induce vasodilatation in human arteries via prostaglandins, nitric oxide and endothelium-derived hyperpolarising factor. Br J Pharmacol 2003; 138:1451–8

44. Coats P, Johnston F, MacDonald J, McMurray JJ, Hillier C: Endothelium-derived hyperpolarizing factor: Identification and mechanisms of action in human subcutaneous resistance arteries. Circulation 2001; 103:1702–8

45. Coleman HA, Tare M, Parkington HC: K+ currents underlying the action of endothelium-derived hyperpolarizing factor in guinea-pig, rat and human blood vessels. J Physiol 2001; 531:359–73

46. Hamilton CA, McPhaden AR, Berg G, Pathi V, Dominiczak AF: Is hydrogen peroxide an EDHF in human radial arteries? Am J Physiol Heart Circ Physiol 2001; 280:H2451–5

47. Matoba T, Shimokawa H, Kubota H, Morikawa K, Fujiki T, Kunihiro I, Mukai Y, Hirakawa Y, Takeshita A: Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in human mesenteric arteries. Biochem Biophys Res Commun 2002; 290:909–13

48. Bodelsson G, Sandstrom K, Wallerstedt SM, Hidestal J, Tornebrandt K, Bodelsson M: Effects of propofol on substance P-induced relaxation in isolated human omental arteries and veins. Eur J Anaesthesiol 2000; 17:720–8

49. Ohlmann P, Martínez MC, Schneider F, Stoclet JC, Andriantsitohaina R: Characterization of endothelium-derived relaxing factors released by bradykinin in human resistance arteries. Br J Pharmacol 1997; 121:657–64

50. Nishikawa Y, Stepp DW, Chilian WM: In vivo location and mechanism of EDHF-mediated vasodilation in canine coronary microcirculation. Am J Physiol 1999; 277:H1252–9

51. Yada T, Shimokawa H, Hiramatsu O, Kajita T, Shigeto F, Goto M, Ogasawara Y, Kajiya F: Hydrogen peroxide, an endogenous endothelium-derived hyperpolarizing factor, plays an important role in coronary autoregulation in vivo. Circulation 2003; 107:1040–5

52. Matsuda H, Hayashi K, Wakino S, Kubota E, Honda M, Tokuyama H, Takamatsu I, Tatematsu S, Saruta T: Role of endothelium-derived hyperpolarizing factor in ACE inhibitor-induced renal vasodilation in vivo. Hypertension 2004; 43:603–9

53. Loeb AL, Gödény I, Longnecker DE: Anesthetics alter relative contributions of NO and EDHF in rat cremaster muscle microcirculation. Am J Physiol Heart Circ Physiol 1997; 273:H618–27

54. Parkington HC, Chow JA, Evans RG, Coleman HA, Tare M: Role for endothelium-derived hyperpolarizing factor in vascular tone in rat mesenteric and hindlimb circulations in vivo. J Physiol 2002; 542:929–37

55. Thomsen K, Rubin I, Lauritzen M: In vivo mechanisms of acetylcholine-induced vasodilation in rat sciatic nerve. Am J Physiol Heart Circ Physiol 2000; 279:H1044–54

56. Passauer J, Bussemaker E, Lassig G, Pistrosch F, Fauler J, Gross P, Fleming I: Baseline blood flow and bradykinin-induced vasodilator responses in the human forearm are insensitive to the cytochrome P450 2C9 (CYP2C9) inhibitor sulphaphenazole. Clin Sci (Lond) 2003; 105:513–8

57. Halcox JPJ, Narayanan S, Cramer-Joyce L, Mincemoyer R, Quyyumi AA: Characterization of endothelium-derived hyperpolarizing factor in the human forearm microcirculation. Am J Physiol Heart Circ Physiol 2001; 280:H2470–7

58. Katz SD, Krum H: Acetylcholine-mediated vasodilation in the forearm circulation of patients with heart failure: Indirect evidence for the role of endothelium-derived hyperpolarizing factor. Am J Cardiol 2001; 87:1089–92

59. McCulloch AI, Randall MD: Sex differences in the relative contributions of nitric oxide and EDHF to agonist-stimulated endothelium-dependent relaxations in the rat isolated mesenteric arterial bed. Br J Pharmacol 1998; 123:1700–6

60. Tep-areenan P, Kendall DA, Randall MD: Testosterone-induced vasorelaxation in the rat mesenteric arterial bed is mediated predominantly via potassium channels. Br J Pharmacol 2002; 135:735–40

61. Liu MY, Hattori Y, Fukao M, Sato A, Sakuma I, Kanno M: Alterations in EDHF-mediated hyperpolarization and relaxation in mesenteric arteries of female rats in long-term deficiency of oestrogen and during oestrus cycle. Br J Pharmacol 2001; 132:1035–46

62. Woodman OL, Boujaoude M: Chronic treatment of male rats with daidzein and 17β-oestradiol induces the contribution of EDHF to endothelium-dependent relaxation. Br J Pharmacol 2004; 141:322–8

63. Golding EM, Kepler TE: Role of estrogen in modulating EDHF-mediated dilations in the female rat middle cerebral artery. Am J Physiol Heart Circ Physiol 2001; 280:H2417–23

64. Golding EM, Ferens DM, Marrelli SP: Altered calcium dynamics do not account for attenuation of endothelium-derived hyperpolarizing factor-mediated dilations in the female middle cerebral artery. Stroke 2002; 33:2972–7

65. Xu HL, Santizo RA, Koenig HM, Pelligrino DA: Chronic estrogen depletion alters adenosine diphosphate-induced pial arteriolar dilation in female rats. Am J Physiol Heart Circ Physiol 2001; 281:H2105–12

66. Xu HL, Santizo RA, Baughman VL, Pelligrino DA: ADP-induced pial arteriolar dilation in ovariectomized rats involves gap junctional communication. Am J Physiol Heart Circ Physiol 2002; 283:H1082–91

67. Xu HL, Santizo RA, Baughman VL, Pelligrino DA: Nascent EDHF-mediated cerebral vasodilation in ovariectomized rats is not induced by eNOS dysfunction. Am J Physiol Heart Circ Physiol 2003; 285:H2045–53

68. Pascoal IF, Lindheimer MD, Nalbantian-Brandt C, Umans JG: Contraction and endothelium-dependent relaxation in mesenteric microvessels from pregnant rats. Am J Physiol 1995; 269:H1899–904

69. Gerber RT, Anwar MA, Poston L: Enhanced acetylcholine induced relaxation in small mesenteric arteries from pregnant rats: An important role for endothelium-derived hyperpolarizing factor (EDHF). Br J Pharmacol 1998; 125: 455–60

70. Pascoal IF, Umans JG: Effect of pregnancy on mechanisms of relaxation in human omental microvessels. Hypertension 1996; 28:183–7

71. McCarthy AL, Taylor P, Graves J, Raju SK, Poston L: Endothelium-dependent relaxation of human resistance arteries in pregnancy. Am J Obstet. Gynecol 1994; 171:1309–15

72. Bobadilla RA, Henkel CC, Henkel EC, Escalante B, Hong E: Possible involvement of endothelium-derived hyperpolarizing factor in vascular responses of abdominal aorta from pregnant rats. Hypertension 1997; 30:596–602

73. Kenny LC, Baker PN, Kendall DA, Randall MD, Dunn WR: Differential mechanisms of endothelium-dependent vasodilator responses in human myometrial small arteries in normal pregnancy and pre-eclampsia. Clin Sci (Lond) 2002; 103:67–73

74. Gillham JC, Kenny LC, Baker PN: An overview of endothelium-derived hyperpolarising factor (EDHF) in normal and compromised pregnancies. Eur J Obstet Gynecol Reprod Biol 2003; 109:2–7

75. Gonzales RJ, Krause DN, Duckles SP: Testosterone suppresses endothelium-dependent dilation of rat middle cerebral arteries. Am J Physiol Heart Circ Physiol 2004; 286:H552–60

76. Bauersachs J, Christ M, Ertl G, Michaelis UR, Fisslthaler B, Busse R, Fleming I: Cytochrome P450 2C expression and EDHF-mediated relaxation in porcine coronary arteries is increased by cortisol. Cardiovasc Res 2002; 54:669–75

77. Marrelli SP: Mechanisms of endothelial P2Y(1)- and P2Y(2)-mediated vasodilatation involve differential [Ca2+]i responses. Am J Physiol Heart Circ Physiol 2001; 281:H1759–66

78. Nagao T, Illiano S, Vanhoutte PM: Heterogeneous distribution of endothelium-dependent relaxations resistant to N^G-nitro-L-arginine in rats. Am J Physiol Heart Circ Physiol 1992; 263:H1090–4

79. Chen GF, Suzuki H: Calcium dependency of the endothelium-dependent hyperpolarization in smooth muscle cells of the rabbit carotid artery. J Physiol 1990; 421:521–34

80. Lagaud GJ, Skarsgard PL, Laher I, Van Breemen C: Heterogeneity of endothelium-dependent vasodilation in pressurized cerebral and small mesenteric resistance arteries of the rat. J Pharmacol Exp Ther 1999; 290:832–9

81. Marrelli SP, Childres WF, Goddard-Finegold J, Bryan RM Jr: Potentiated EDHF-mediated dilations in the rat middle cerebral artery following ischemia/reperfusion, EDHF 2000. Edited by Vanhoutte PM. New York, Taylor and Francis, 2001, pp 388–99

82. Taylor HJ, Chaytor AT, Edwards DH, Griffith TM: Gap junction-dependent increases in smooth muscle cAMP underpin the EDHF phenomenon in rabbit arteries. Biochem Biophys Res Commun 2001; 283:583–9

83. Tomioka H, Hattori Y, Fukao M, Watanabe H, Akaishi Y, Sato A, Kim TQ, Sakuma I, Kitabatake A, Kanno M: Role of endothelial Ni2+-sensitive Ca2+ entry pathway in regulation of EDHF in porcine coronary artery. Am J Physiol Heart Circ Physiol 2001; 280:H730–7

84. You J, Marrelli SP, Bryan Jr RM: Role of cytoplasmic phospholipase A2 in endothelium-derived hyperpolarizing factor dilations of rat middle cerebral arteries. J Cereb Blood Flow Metab 2002; 22:1239–47

85. Weintraub NL, Stephenson AH, Sprague RS, McMurdo L, Lonigro AJ: Relationship of arachidonic acid release to porcine coronary artery relaxation. Hypertension 1995; 26:684–90

86. Fukao M, Hattori Y, Kanno M, Sakuma I, Kitabatake A: Sources of Ca^{2+} in relation to generation of acetylcholine-induced endothelium-dependent hyperpolarization in rat mesenteric artery. Br J Pharmacol 1997; 120:1328–34

87. Davis MJ, Sharma NR: Calcium-release-activated calcium influx in endothelium. J Vasc Res 1997; 34:186–95

88. Bryan RM Jr, Steenberg ML, Marrelli SP: Role of endothelium in shear stress-induced constrictions in rat middle cerebral artery. Stroke 2001; 32:1394–400

89. Marrelli SP: Selective measurement of endothelial or smooth muscle $[Ca^{2+}]_i$ in pressurized/perfused cerebral arteries with fura-2. J Neurosci Methods 2000; 97:145–55

90. Marrelli SP: Altered endothelial Ca2+ regulation after ischemia/reperfusion produces potentiated endothelium-derived hyperpolarizing factor-mediated dilations. Stroke 2002; 33:2285–91

91. Chauhan SD, Nilsson H, Ahluwalia A, Hobbs AJ: Release of C-type natriuretic peptide accounts for the biological activity of endothelium-derived hyperpolarizing factor. Proc Natl Acad Sci U S A 2003; 100:1426–31

92. Randall MD, Alexander SPH, Bennett T, Boyd EA, Fry JR, Gardiner SM, Kemp PA, McCulloch AI, Kendall DA: An endogenous cannabinoid as an endothelium-derived vasorelaxant. Biochem Biophys Res Commun 1996; 229:114–20

93. Randall MD, McCulloch AI, Kendall DA: Comparative pharmacology of endothelium-derived hyperpolarizing factor and anandamide in rat isolated mesentery. Eur J Pharmacol 1997; 333:191–7

94. Randall MD, Kendall DA: Anandamide and endothelium-derived hyperpolarizing factor act via a common vasorelaxant mechanism in rat mesentery. Eur J Pharmacol 1998; 346:51–3

95. Channon JY, Leslie CC: A calcium-dependent mechanism for associating a soluble arachidonoyl-hydrolyzing phospholipase A2 with membrane in the macrophage cell line RAW 264.7. J Biol Chem 1990; 265:5409–13

96. Adeagbo AS, Henzel MK: Calcium-dependent phospholipase A2 mediates the production of endothelium-derived hyperpolarizing factor in perfused rat mesenteric prearteriolar bed. J Vasc Res 1998; 35:27–35

97. Fulton D, McGiff JC, Quilley J: Role of phospholipase C and phospholipase A2 in the nitric oxide-independent vasodilator effect of bradykinin in the rat perfused heart. J Pharmacol Exp Ther 1996; 278:518–26

98. Bolz SS, Fisslthaler B, Pieperhoff S, De Wit C, Fleming I, Busse R, Pohl U: Antisense oligonucleotides against cytochrome P450 2C8 attenuate EDHF-mediated Ca(2+) changes and dilation in isolated resistance arteries. FASEB J 2000; 14:255–60

99. Bauersachs J, Hecker M, Busse R: Display of the characteristics of endothelium-derived hyperpolarizing factor by a cytochrome P450-derived arachidonic acid metabolite in the coronary microcirculation. Br J Pharmacol 1994; 113:1548–53

100. Imig JD, Falck JR, Wei SZ, Capdevila JH: Epoxygenase metabolites contribute to nitric oxide-independent afferent arteriolar vasodilation in response to bradykinin. J Vasc Res 2001; 38:247–55

101. Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I, Busse R: Cytochrome P450 2C is an EDHF synthase in coronary arteries. Nature 1999; 401:493–7

102. Campbell WB, Gebremedhin D, Pratt PF, Harder DR: Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. Circ Res 1996; 78:415–23

103. Gauthier KM, Deeter C, Krishna UM, Reddy YK, Bondlela M, Falck JR, Campbell WB: 14,15-Epoxyeicosa-5(Z)-enoic acid: A selective epoxyeicosatrienoic acid antagonist that inhibits endothelium-dependent hyperpolarization and relaxation in coronary arteries. Circ Res 2002; 90:1028–36

104. Rosolowsky M, Campbell WB: Synthesis of hydroxyeicosatetraenoic (HETEs) and epoxyeicosatrienoic acids (EETs) by cultured bovine coronary artery endothelial cells. Biochim Biophys Acta 1996; 1299:267–77

105. Gebremedhin D, Ma YH, Falck JR, Roman RJ, VanRollins M, Harder DR: Mechanism of action of cerebral epoxyeicosatrienoic acids on cerebral arterial smooth muscle. Am J Physiol 1992; 263:H519–25

106. Popp R, Bauersachs J, Hecker M, Fleming I, Busse R: A transferable, beta-naphthoflavone-inducible, hyperpolarizing factor is synthesized by native and cultured porcine coronary endothelial cells. J Physiol 1996; 497:699–709

107. Gebremedhin D, Harder DR, Pratt PF, Campbell WB: Bioassay of an endothelium-derived hyperpolarizing factor from bovine coronary arteries: Role of a cytochrome P450 metabolite. J Vasc Res 1998; 35:274–84

108. Busse R, Edwards G, Feletou M, Fleming I, Vanhoutte PM, Weston AH: EDHF: Bringing the concepts together. Trends Pharmacol Sci 2002; 23:374–80

109. Hecker M, Bara AT, Bauersachs J, Busse R: Characterization of endothelium-derived hyperpolarizing factor as a cytochrome P450-derived arachidonic acid metabolite in mammals. J Physiol (Lond) 1994; 481(pt 2):407–14

110. Petersson J, Zygmunt PM, Jonsson P, Hogestatt ED: Characterization of

endothelium-dependent relaxation in guinea pig basilar artery: Effect of hypoxia and role of cytochrome P450 mono-oxygenase. J Vasc Res 1998; 35:285–94

111. Zygmunt PM, Edwards G, Weston AH, Davis SC, Högestätt ED: Effects of cytochrome P450 inhibitors on EDHF-mediated relaxation in the rat hepatic artery. Br J Pharmacol 1996; 118:1147–52

112. Vanheel B, Van de Voorde J: Evidence against the involvement of cytochrome P450 metabolites in endothelium-dependent hyperpolarization of the rat main mesenteric artery. J Physiol (Lond) 1997; 501:331–41

113. Richards CF, Johnson AR, Campbell WB: Specific incorporation of 5-hydroxy-6,8,11,14-eicosatetraenoic acid into phosphatidylcholine in human endothelial cells. Biochim Biophys Acta 1986; 875:569–81

114. VanRollins M, Kaduce TL, Knapp HR, Spector AA: 14,15-Epoxyeicosatrienoic acid metabolism in endothelial cells. J Lipid Res 1993; 34:1931–42

115. VanRollins M, Kaduce TL, Fang X, Knapp HR, Spector AA: Arachidonic acid diols produced by cytochrome P-450 monooxygenases are incorporated into phospholipids of vascular endothelial cells. J Biol Chem 1996; 271:14001–9

116. Weintraub NL, Fang X, Kaduce TL, VanRollins M, Chatterjee P, Spector AA: Epoxide hydrolases regulate epoxyeicosatrienoic acid incorporation into coronary endothelial phospholipids. Am J Physiol 1999; 277:H2098–108

117. Weintraub NL, Fang X, Kaduce TL, VanRollins M, Chatterjee P, Spector AA: Potentiation of endothelium-dependent relaxation by epoxyeicosatrienoic acids. Circ Res 1997; 81:258–67

118. Mombouli JV, Ntsikoussalabongui B, Ballard K, Orskiszewski R, Taylor AA, Vanhoutte PM: Phospholipid-derived epoxyeicosatrienoic acids mediate the relaxations attributed to endothelium-derived hyperpolarizing factor, Endothelium-dependent Hyperpolarizations. Edited by Vanhoutte PM. Amsterdam, Harwood, 1996, pp 29–39

119. Fisslthaler B, Hinsch N, Chataigneau T, Popp R, Kiss L, Busse R, Fleming I: Nifedipine increases cytochrome P4502C expression and endothelium-derived hyperpolarizing factor-mediated responses in coronary arteries. Hypertension 2000; 36:270–5

120. Zygmunt PM, Högestätt ED: Role of potassium channels in endothelium-dependent relaxation resistant to nitroarginine in the rat hepatic artery. Br J Pharmacol 1996; 117:1600–6

121. Fleming I: Cytochrome P450 epoxygenases as EDHF synthase(s). Pharmacol Res 2004; 49:525–33

122. Rzigalinski BA, Willoughby KA, Hoffman SW, Falck JR, Ellis EF: Calcium influx factor, further evidence it is 5, 6-epoxyeicosatrienoic acid. J Biol Chem 1999; 274:175–82

123. Graier WF, Simecek S, Sturek M: Cytochrome P450 mono-oxygenase-regulated signalling of Ca2+ entry in human and bovine endothelial cells. J Physiol 1995; 482(pt 2):259–74

124. Mombouli JV, Holzmann S, Kostner GM, Graier WF: Potentiation of Ca2+ signaling in endothelial cells by 11,12-epoxyeicosatrienoic acid. J Cardiovasc Pharmacol 1999; 33:779–84

125. Baron A, Frieden M, Beny JL: Epoxyeicosatrienoic acids activate a high-conductance, Ca(2+)-dependent K + channel on pig coronary artery endothelial cells. J Physiol (Lond) 1997; 504:537–43

126. Edwards G, Thollon C, Gardener MJ, Feletou M, Vilaine J, Vanhoutte PM, Weston AH: Role of gap junctions and EETs in endothelium-dependent hyperpolarization of porcine coronary artery. Br J Pharmacol 2000; 129:1145–54

127. Popp R, Brandes RP, Ott G, Busse R, Fleming I: Dynamic modulation of interendothelial gap junctional communication by 11,12-epoxyeicosatrienoic acid. Circ Res 2002; 90:800–6

128. Roman RJ: P-450 metabolites of arachidonic acid in the control of cardiovascular function. Phys Rev 2002; 82:131–85

129. Pfister SL, Spitzbarth N, Edgemond W, Campbell WB: Vasorelaxation by an endothelium-derived metabolite of arachidonic acid. Am J Physiol 1996; 270:H1021–30

130. Pfister SL, Spitzbarth N, Nithipatikom K, Edgemond WS, Falck JR, Campbell WB: Identification of the 11,14,15- and 11,12, 15-trihydroxyeicosatrienoic acids as endothelium-derived relaxing factors of rabbit aorta. J Biol Chem 1998; 273:30879–87

131. Faraci FM, Sobey CG, Chrissobolis S, Lund DD, Heistad DD, Weintraub NL: Arachidonate dilates basilar artery by lipoxygenase-dependent mechanism and activation of K+ channels. Am J Physiol Regulatory Integrative Comp Physiol 2001; 281:R246–53

132. Campbell WB, Spitzbarth N, Gauthier KM, Pfister SL: 11,12,15-Trihydroxyeicosatrienoic acid mediates ACh-induced relaxations in rabbit aorta. Am J Physiol Heart Circ Physiol 2003; 285:H2648–56

133. Bradley KK, Jaggar JH, Bonev AD, Heppner TJ, Flynn ER, Nelson MT, Horowitz B: Kir2.1 encodes the inward rectifier potassium channel in rat arterial smooth muscle cells. J Physiol 1999; 515:639–51

134. Knot HJ, Zimmermann PA, Nelson MT: Extracellular K^+-induced hyperpolarizations and dilatations of rat coronary and cerebral arteries involve inward rectifier K^+ channels. J Physiol 1996; 492:419–30

135. McCarron JG, Halpern W: Potassium dilates rat cerebral arteries by two independent mechanisms. Am J Physiol 1990; 259:H902–8

136. Johnson TD, Marrelli SP, Steenberg ML, Childres WF, Bryan RM Jr: Inward rectifier potassium channels in the rat middle cerebral artery. Am J Physiol 1998; 274:R541–7

137. Marrelli SP, Johnson TD, Khorovets A, Childres WF, Bryan RM Jr: Altered function of inward rectifier potassium channels in cerebrovascular smooth muscle after ischemia/reperfusion. Stroke 1998; 29:1469–74

138. Blanco G, Mercer RW: Isozymes of the Na-K-ATPase: Heterogeneity in structure, diversity in function. Am J Physiol 1998; 275:F633–50

139. Weston AH, Richards GR, Burnham MP, Feletou M, Vanhoutte PM, Edwards G: K+-induced hyperpolarization in rat mesenteric artery: Identification, localization and role of Na+/K+-ATPases. Br J Pharmacol 2002; 136:918–26

140. Edwards G, Gardener MJ, Feletou M, Brady G, Vanhoutte PM, Weston AH: Further investigation of endothelium-derived hyperpolarizing factor (EDHF) in rat hepatic artery: Studies using 1-EBIO and ouabain. Br J Pharmacol 1999; 128:1064–70

141. Edwards G, Dora KA, Gardener MJ, Garland CJ, Weston AH: K+ is an endothelium-derived hyperpolarizing factor in rat arteries. Nature 1998; 396: 269–72

142. Doughty JM, Plane F, Langton PD: Charybdotoxin and apamin block EDHF in rat mesenteric artery if selectively applied to the endothelium. Am J Physiol 1999; 276:H1107–12

143. Eichler I, Wibawa J, Grgic I, Knorr A, Brakemeier S, Pries AR, Hoyer J, Kohler R: Selective blockade of endothelial Ca2+-activated small- and intermediate-conductance K+-channels suppresses EDHF-mediated vasodilation. Br J Pharmacol 2003; 138:594–601

144. Burnham MP, Bychkov R, Feletou M, Richards GR, Vanhoutte PM, Weston AH, Edwards G: Characterization of an apamin-sensitive small-conductance Ca2+-activated K+ channel in porcine coronary artery endothelium: Relevance to EDHF. Br J Pharmacol 2002; 135:1133–43

145. Bychkov R, Burnham MP, Richards GR, Edwards G, Weston AH, Feletou M, Vanhoutte PM: Characterization of a charybdotoxin-sensitive intermediate conductance Ca2+-activated K+ channel in porcine coronary endothelium: Relevance to EDHF. Br J Pharmacol 2002; 137:1346–54

146. Vanheel B, Van de Voorde J: Barium decreases endothelium-dependent smooth muscle responses to transient but not to more prolonged acetylcholine applications. Pflugers Arch 1999; 439:123–9

147. Andersson DA, Zygmunt PM, Movahed P, Andersson TL, Hogestatt ED: Effects of inhibitors of small- and intermediate-conductance calcium- activated potassium channels, inwardly-rectifying potassium channels and Na(+)/K(+) ATPase on EDHF relaxations in the rat hepatic artery. Br J Pharmacol 2000; 129:1490–6

148. Zygmunt PM, Sorgard M, Petersson J, Johansson R, Hogestatt ED: Differential actions of anandamide, potassium ions and endothelium-derived hyperpolarizing factor in guinea-pig basilar artery. Naunyn Schmiedebergs Arch Pharmacol 2000; 361:535–42

149. Ding H, Kubes P, Triggle C: Potassium- and acetylcholine-induced vasorelaxation in mice lacking endothelial nitric oxide synthase. Br J Pharmacol 2000; 129:1194–200

150. Quignard JF, Feletou M, Thollon C, Vilaine JP, Duhault J, Vanhoutte PM: Potassium ions and endothelium-derived hyperpolarizing factor in guinea-pig carotid and porcine coronary arteries. Br J Pharmacol 1999; 127:27–34

151. Drummond GR, Selemidis S, Cocks TM: Apamin-sensitive, non-nitric oxide (NO) endothelium-dependent relaxations to bradykinin in the bovine isolated coronary artery: No role for cytochrome P(450) and K. Br J Pharmacol 2000; 129:811–9

152. Lacy PS, Pilkington G, Hanvesakul R, Fish HJ, Boyle JP, Thurston H: Evidence against potassium as an endothelium-derived hyperpolarizing factor in rat mesenteric small arteries. Br J Pharmacol 2000; 129:605–11

153. Richards GR, Weston AH, Burnham MP, Feletou M, Vanhoutte PM, Edwards G: Suppression of K+-induced hyperpolarization by phenylephrine in rat mesenteric artery: Relevance to studies of endothelium-derived hyperpolarizing factor. Br J Pharmacol 2001; 134:1–5

154. Dora KA, Garland CJ: Properties of smooth muscle hyperpolarization and relaxation to K+ in the rat isolated mesenteric artery. Am J Physiol Heart Circ Physiol 2001; 280:H2424–9

155. Beblo DA, Wang HZ, Beyer EC, Westphale EM, Veenstra RD: Unique conductance, gating, and selective permeability properties of gap junction channels formed by connexin40. Circ Res 1995; 77:813–22

156. Wang HZ, Veenstra RD: Monovalent ion selectivity sequences of the rat connexin43 gap junction channel. J Gen Physiol 1997; 109:491–507

157. Christ GJ, Brink PR: Analysis of the presence and physiological relevance of subconducting states of Connexin43-derived gap junction channels in cultured human corporal vascular smooth muscle cells. Circ Res 1999; 84:797–803

158. Christ GJ, Spray DC, el Sabban M, Moore LK, Brink PR: Gap junctions in vascular tissues: Evaluating the role of intercellular communication in the modulation of vasomotor tone. Circ Res 1996; 79:631–46

159. Churchill GC, Lurtz MM, Louis CF: Ca2+ regulation of gap junctional coupling in lens epithelial cells. Am J Physiol Cell Physiol 2001; 281:C972–81

160. Dora KA, Doyle MP, Duling BR: Elevation of intracellular calcium in smooth muscle causes endothelial cell generation of NO in arterioles. Proc Natl Acad Sci U S A 1997; 94:6529–34

161. Brink PR, Ricotta J, Christ GJ: Biophysical characteristics of gap junctions in vascular wall cells: Implications for vascular biology and disease. Braz J Med Biol Res 2000; 33:415–22

162. Veenstra RD, Wang HZ, Beblo DA, Chilton MG, Harris AL, Beyer EC, Brink PR: Selectivity of connexin-specific gap junctions does not correlate with channel conductance. Circ Res 1995; 77:1156–65

163. Sandow SL, Hill CE: Incidence of myoendothelial gap junctions in the proximal and distal mesenteric arteries of the rat is suggestive of a role in endothelium-derived hyperpolarizing factor-mediated responses. Circ Res 2000; 86:341–6

164. Emerson GG, Segal SS: Electrical activation of endothelium evokes vasodilation and hyperpolarization along hamster feed arteries. Am J Physiol Heart Circ Physiol 2001; 280:H160–7

165. Emerson GG, Segal SS: Electrical coupling between endothelial cells and smooth muscle cells in hamster feed arteries: Role in vasomotor control. Circ Res 2000; 87:474–9

166. Hill CE, Phillips JK, Sandow SL: Heterogeneous control of blood flow amongst different vascular beds. Med Res Rev 2001; 21:1–60

167. Chaytor AT, Evans WH, Griffith TM: Central role of heterocellular gap junctional communication in endothelium-dependent relaxations of rabbit arteries. J Physiol (Lond) 1998; 508:561–73

168. Chaytor AT, Martin PEM, Edwards DH, Griffith TM: Gap junctional communication underpins EDHF-type relaxations evoked by ACh in the rat hepatic artery. Am J Physiol Heart Circ Physiol 2001; 280:H2441–50

169. Dora KA, Martin PM, Chaytor AT, Evans WH, Garland CJ, Griffith TM: Role of heterocellular Gap junctional communication in endothelium-dependent smooth muscle hyperpolarization: Inhibition by a connexin-mimetic peptide. Biochem Biophys Res Commun 1999; 254:27–31

170. Ujiie H, Chaytor AT, Bakker LM, Griffith TM: Essential role of gap junctions in NO- and prostanoid-independent relaxations evoked by acetylcholine in rabbit intracerebral arteries. Stroke 2003; 34:544–50

171. Sandow SL, Tare M, Coleman HA, Hill CE, Parkington HC: Involvement of myoendothelial gap junctions in the actions of endothelium-derived hyperpolarizing factor. Circ Res 2002; 90:1108–13

172. Leybaert L, Braet K, Vandamme W, Cabooter L, Martin PE, Evans WH: Connexin channels, connexin mimetic peptides and ATP release. Cell Commun Adhes 2003; 10:251–7

173. Fleming I: Myoendothelial gap junctions: The gap is there, but does EDHF go through it? Circ Res 2000; 86:249–50

174. Coleman HA, Tare M, Parkington HC: EDHF is not K+ but may be due to spread of current from the endothelium in guinea pig arterioles. Am J Physiol Heart Circ Physiol 2001; 280:H2478–83

175. Lacza Z, Puskar M, Kis B, Perciaccante JV, Miller AW, Busija DW: Hydrogen peroxide acts as an EDHF in the piglet pial vasculature in response to bradykinin. Am J Physiol Heart Circ Physiol 2002; 283:H406–11

176. Matoba T, Shimokawa H, Nakashima M, Hirakawa Y, Mukai Y, Hirano K, Kanaide H, Takeshita A: Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. J Clin Invest 2000; 106:1521–30

177. Matoba T, Shimokawa H, Morikawa K, Kubota H, Kunihiro I, Urakami-Harasawa L, Mukai Y, Hirakawa Y, Akaike T, Takeshita A: Electron spin resonance detection of hydrogen peroxide as an endothelium-derived hyperpolarizing factor in porcine coronary microvessels. Arterioscler Thromb Vasc Biol 2003; 23:1224–30

178. Sobey CG, Heistad DD, Faraci FM: Mechanisms of bradykinin-induced cerebral vasodilatation in rats: Evidence that reactive oxygen species activate K+ channels. Stroke 1997; 28:2290–4

179. Sobey CG, Heistad DD, Faraci FM: Potassium channels mediate dilatation of cerebral arterioles in response to arachidonate. Am J Physiol 1998; 275: H1606–12

180. Yang ST, Mayhan WG, Faraci FM, Heistad DD: Mechanisms of impaired endothelium-dependent cerebral vasodilatation in response to bradykinin in hypertensive rats. Stroke 1991; 22:1177–82

181. Wei EP, Kontos HA, Beckman JS: Mechanisms of cerebral vasodilation by superoxide, hydrogen peroxide, and peroxynitrite. Am J Physiol 1996; 271: H1262–6

182. Bharadwaj L, Prasad K: Mediation of H2O2-induced vascular relaxation by endothelium-derived relaxing factor. Mol Cell Biochem 1995; 149–150:267–70

183. Barlow RS, White RE: Hydrogen peroxide relaxes porcine coronary arteries by stimulating BKCa channel activity. Am J Physiol Heart Circ Physiol 1998; 275:H1283–9

184. Fleming I, Michaelis UR, Bredenkotter D, Fisslthaler B, Dehghani F, Brandes RP, Busse R: Endothelium-derived hyperpolarizing factor synthase (cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries. Circ Res 2001; 88:44–51

185. Liu Y, Zhao H, Li H, Kalyanaraman B, Nicolosi AC, Gutterman DD: Mitochondrial sources of H2O2 generation play a key role in flow-mediated dilation in human coronary resistance arteries. Circ Res 2003; 93:573–80

186. Pomposiello S, Rhaleb NE, Alva M, Carretero OA: Reactive oxygen species: Role in the relaxation induced by bradykinin or arachidonic acid via EDHF in isolated porcine coronary arteries. J Cardiovasc Pharmacol 1999; 34:567–74

187. Ellis A, Pannirselvam M, Anderson TJ, Triggle CR: Catalase has negligible inhibitory effects on endothelium-dependent relaxations in mouse isolated aorta and small mesenteric artery. Br J Pharmacol 2003; 140:1193–200

188. Tanaka M, Kanatsuka H, Ong BH, Tanikawa T, Uruno A, Komaru T, Koshida R, Shirato K: Cytochrome P-450 metabolites but not NO, PGI2, and H2O2 contribute to ACh-induced hyperpolarization of pressurized canine coronary microvessels. Am J Physiol Heart Circ Physiol 2003; 285:H1939–48

189. Chaytor AT, Edwards DH, Bakker LM, Griffith TM: Distinct hyperpolarizing and relaxant roles for gap junctions and endothelium-derived H2O2 in NO-independent relaxations of rabbit arteries. Proc Natl Acad Sci U S A 2003; 100:15212–7

190. Cosentino F, Patton S, d'Uscio LV, Werner ER, Werner-Felmayer G, Moreau P, Malinski T, Luscher TF: Tetrahydrobiopterin alters superoxide and nitric oxide release in prehypertensive rats. J Clin Invest 1998; 101:1530–7

191. Cai H, Griendling KK, Harrison DG: The vascular NAD(P)H oxidases as therapeutic targets in cardiovascular diseases. Trends Pharmacol Sci 2003; 24: 471–8

192. Katusic ZS: Vascular endothelial dysfunction: Does tetrahydrobiopterin play a role? Am J Physiol Heart Circ Physiol 2001; 281:H981–6

193. Hink U, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M, Skatchkov M, Thaiss F, Stahl RA, Warnholtz A, Meinertz T, Griendling K, Harrison DG, Forstermann U, Munzel T: Mechanisms underlying endothelial dysfunction in diabetes mellitus. Circ Res 2001; 88:E14–22

194. Cherian L, Chacko G, Goodman JC, Robertson CS: Cerebral hemodynamic effects of phenylephrine and L-arginine after cortical impact injury. Crit Care Med 1999; 27:2512–7

195. Cherian L, Goodman JC, Robertson CS: Brain nitric oxide changes after controlled cortical impact injury in rats. J Neurophysiol 2000; 83:2171–8

196. Kaw S, Hecker M: Endothelium-derived hyperpolarizing factor, but not nitric oxide or prostacyclin release, is resistant to menadione-induced oxidative stress in the bovine coronary artery. Naunyn Schmiedebergs Arch Pharmacol 1999; 359:133–9

197. Golding EM, You J, Robertson CS, Bryan RM Jr: Potentiated endothelium-derived hyperpolarizing factor-mediated dilations in cerebral arteries following mild head injury. J Neurotrauma 2001; 18:691–7

198. Marrelli SP, Khorovets A, Johnson TD, Childres WF, Bryan RM Jr: P2 purinoceptor-mediated dilations in the rat middle cerebral artery after ischemia/reperfusion. Am J Physiol 1999; 276:H33–41

199. Malmsjo M, Bergdahl A, Zhao XH, Sun XY, Hedner T, Edvinsson L, Erlinge D: Enhanced acetylcholine and P2Y-receptor stimulated vascular EDHF-dilatation in congestive heart failure. Cardiovasc Res 1999; 43:200–9

200. Brandes RP, Behra A, Lebherz C, Boger RH, Bode-Boger SM, Phivthongngam L, Mugge A: N(G)-nitro-L-arginine- and indomethacin-resistant endothelium-dependent relaxation in the rabbit renal artery: Effect of hypercholesterolemia. Atherosclerosis 1997; 135:49–55

201. Miura H, Wachtel RE, Liu Y, Loberiza FR Jr, Saito T, Miura M, Gutterman DD: Flow-induced dilation of human coronary arterioles: Important role of Ca2+-activated K+ channels. Circulation 2001; 103:1992–8

202. Thollon C, Fournet-Bourguignon MP, Saboureau D, Lesage L, Reure H, Vanhoutte PM, Vilaine JP: Consequences of reduced production of NO on vascular reactivity of porcine coronary arteries after angioplasty: Importance of EDHF. Br J Pharmacol 2002; 136:1153–61

203. Chan EC, Woodman OL: Enhanced role for the opening of potassium channels in relaxant responses to acetylcholine after myocardial ischaemia and reperfusion in dog coronary arteries. Br J Pharmacol 1999; 126:925–32

204. Bussemaker E, Popp R, Fisslthaler B, Larson CM, Fleming I, Busse R, Brandes RP: Hyperthyroidism enhances endothelium-dependent relaxation in the rat renal artery. Cardiovasc Res 2003; 59:181–8

205. Bussemaker E, Popp R, Fisslthaler B, Larson CM, Fleming I, Busse R, Brandes RP: Aged spontaneously hypertensive rats exhibit a selective loss of EDHF-mediated relaxation in the renal artery. Hypertension 2003; 42:562–8

206. Randall MD, March JE: Characterization of endothelium-dependent relaxations in mesenteries from transgenic hypertensive rats. Eur J Pharmacol 1998; 358:31–40

207. Sofola OA, Knill A, Hainsworth R, Drinkhill M: Change in endothelial function in mesenteric arteries of Sprague-Dawley rats fed a high salt diet. J Physiol 2002; 543:255–60

208. Fujii K, Tominaga M, Ohmori S, Kobayashi K, Koga T, Takata Y, Fujishima M: Decreased endothelium-dependent hyperpolarization to acetylcholine in smooth muscle of the mesenteric artery of spontaneously hypertensive rats. Circ Res 1992; 70:660–9

209. Goto K, Fujii K, Onaka U, Abe I, Fujishima M: Renin-angiotensin system blockade improves endothelial dysfunction in hypertension. Hypertension 2000; 36:575–80

210. Mantelli L, Amerini S, Ledda F: Roles of nitric oxide and endothelium-derived hyperpolarizing factor in vasorelaxant effect of acetylcholine as influenced by aging and hypertension. J Cardiovasc Pharmacol 1995; 25:595–602

211. Onaka U, Fujii K, Abe I, Fujishima M: Antihypertensive treatment improves endothelium-dependent hyperpolarization in the mesenteric artery of spontaneously hypertensive rats. Circulation 1998; 98:175–82

212. Kemp BK, Smolich JJ, Ritchie BC, Cocks TM: Endothelium-dependent relaxations in sheep pulmonary arteries and veins: Resistance to block by NG-nitro-L-arginine in pulmonary hypertension. Br J Pharmacol 1995; 116:2457–67

213. Fujii K, Ohmori S, Tominaga M, Abe I, Takata Y, Ohya Y, Kobayashi K, Fujishima M: Age-related changes in endothelium-dependent hyperpolarization in the rat mesenteric artery. Am J Physiol Heart Circ Physiol 1993; 265:H509–16

214. Jack AM, Keegan A, Cotter MA, Cameron NE: Effects of diabetes and evening primrose oil treatment on responses of aorta, corpus cavernosum and mesenteric vasculature in rats. Life Sci 2002; 71:1863–77

215. Wigg SJ, Tare M, Tonta MA, O'Brien RC, Meredith IT, Parkington HC: Comparison of effects of diabetes mellitus on an EDHF-dependent and an EDHF-independent artery. Am J Physiol Heart Circ Physiol 2001; 281:H232–40

216. Fukao M, Hattori Y, Kanno M, Sakuma I, Kitabatake A: Alterations in endothelium-dependent hyperpolarization and relaxation in mesenteric arteries from streptozotocin-induced diabetic rats. Br J Pharmacol 1997; 121:1383–91
217. Keegan A, Jack AM, Cotter MA, Cameron NE: Effects of aldose reductase inhibition on responses of the corpus cavernosum and mesenteric vascular bed of diabetic rats. J Cardiovasc Pharmacol 2000; 35:606–13
218. Makino A, Ohuchi K, Kamata K: Mechanisms underlying the attenuation of endothelium-dependent vasodilatation in the mesenteric arterial bed of the streptozotocin-induced diabetic rat. Br J Pharmacol 2000; 130:549–56
219. Matsumoto T, Kobayashi T, Kamata K: Alterations in EDHF-type relaxation and phosphodiesterase activity in mesenteric arteries from diabetic rats. Am J Physiol Heart Circ Physiol 2003; 285:H283–91
220. Minami A, Ishimura N, Harada N, Sakamoto S, Niwa Y, Nakaya Y: Exercise training improves acetylcholine-induced endothelium-dependent hyperpolarization in type 2 diabetic rats, Otsuka Long-Evans Tokushima fatty rats. Atherosclerosis 2002; 162:85–92
221. Pannirselvam M, Anderson TJ, Triggle CR: Characterization of endothelium-derived hyperpolarizing factor-mediated relaxation of small mesenteric arteries from diabetic (db/db -/-) mice, EDHF 2002. Edited by Vanhoutte PM. New York, Taylor and Francis, 2003, pp 124–31
222. Fleming I, Busse R: Vascular cytochrome P450 in the regulation of renal function and vascular tone: EDHF, superoxide anions and blood pressure. Nephrol Dial Transplant 2001; 16:1309–11
223. Brandes RP, Schmitz-Winnenthal FH, Feletou M, Godecke A, Huang PL, Vanhoutte PM, Fleming I, Busse R: An endothelium-derived hyperpolarizing factor distinct from NO and prostacyclin is a major endothelium-dependent vasodilator in resistance vessels of wild-type and endothelial NO synthase knockout mice. Proc Natl Acad Sci U S A 2000; 97:9747–52
224. Huang A, Sun D, Smith CJ, Connetta JA, Shesely EG, Koller A, Kaley G: In eNOS knockout mice skeletal muscle arteriolar dilation to acetylcholine is mediated by EDHF. Am J Physiol Heart Circ Physiol 2000; 278:H762–8
225. Faraci FM, Sigmund CD, Shesely EG, Maeda N, Heistad DD: Responses of carotid artery in mice deficient in expression of the gene for endothelial NO synthase. Am J Physiol 1998; 274:H564–70
226. Chataigneau T, Feletou M, Huang PL, Fishman MC, Duhault J, Vanhoutte PM: Acetylcholine-induced relaxation in blood vessels from endothelial nitric oxide synthase knockout mice. Br J Pharmacol 1999; 126:219–26
227. Waldron GJ, Ding H, Lovren F, Kubes P, Triggle CR: Acetylcholine-induced relaxation of peripheral arteries isolated from mice lacking endothelial nitric oxide synthase. Br J Pharmacol 1999; 128:653–8
228. Faraci FM, Lynch C, Lamping KG: Responses of cerebral arterioles to ADP: eNOS-dependent and eNOS-independent mechanisms. Am J Physiol Heart Circ Physiol 2004; 278:H2871–6
229. Najibi S, Cowan CL, Palacino JJ, Cohen RA: Enhanced role of potassium channels in relaxations to acetylcholine in hypercholesterolemic rabbit carotid artery. Am J Physiol 1994; 266:H2061–7
230. Kagota S, Tamashiro A, Yamaguchi Y, Nakamura K, Kunitomo M: Excessive salt or cholesterol intake alters the balance among endothelium-derived factors released from renal arteries in spontaneously hypertensive rats. J Cardiovasc Pharmacol 1999; 34:533–9
231. Hecker M: Endothelium-derived hyperpolarizing factor: Fact or fiction? NIPS 2000; 15:1–5
232. Lischke V, Busse R, Hecker M: Inhalation anesthetics inhibit the release of endothelium-derived hyperpolarizing factor in the rabbit carotid artery. Anesthesiology 1995; 83:574–82
233. Lischke V, Busse R, Hecker M: Volatile and intravenous anesthetics selectively attenuate the release of endothelium-derived hyperpolarizing factor elicited by bradykinin in the coronary microcirculation. Naunyn Schmiedebergs Arch Pharmacol 1995; 352:346–9
234. Lischke V, Busse R, Hecker M: Selective inhibition by barbiturates of the synthesis of endothelium-derived hyperpolarizing factor in the rabbit carotid artery. Br J Pharmacol 1995; 115:969–74
235. Peracchia C: Effects of the anesthetics heptanol, halothane and isoflurane on gap junction conductance in crayfish septate axons: A calcium- and hydrogen-independent phenomenon potentiated by caffeine and theophylline, and inhibited by 4-aminopyridine. J Membr Biol 1991; 121:67–78
236. He DS, Burt JM: Mechanism and selectivity of the effects of halothane on gap junction channel function. Circ Res 2000; 86:E104–9
237. Burt JM, Spray DC: Volatile anesthetics block intercellular communication between neonatal rat myocardial cells. Circ Res 1989; 65:829–37
238. Petros A, Bennett D, Vallance P: Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. Lancet 1991; 338:1557–8
239. Vallance P, Chan N: Endothelial function and nitric oxide: Clinical relevance. Heart 2001; 85:342–50
240. Keefer LK: Progress toward clinical application of the nitric oxide-releasing diazeniumdiolates. Annu Rev Pharmacol Toxicol 2003; 43:585–607
241. Shah V, Kamath PS: Nitric oxide in liver transplantation: Pathobiology and clinical implications. Liver Transpl 2003; 9:1–11
242. Thomas GD, Zhang W, Victor RG: Nitric oxide deficiency as a cause of clinical hypertension: Promising new drug targets for refractory hypertension. JAMA 2001; 285:2055–7
243. Grant MK, El Fakahany EE: Therapeutic interventions targeting the nitric oxide system: Current and potential uses in obstetrics, bone disease and erectile dysfunction. Life Sci 2004; 74:1701–21
244. Reiter CD, Gladwin MT: An emerging role for nitric oxide in sickle cell disease vascular homeostasis and therapy. Curr Opin Hematol 2003; 10:99–107
245. Macrae DJ, Field D, Mercier JC, Moller J, Stiris T, Biban P, Cornick P, Goldman A, Gothberg S, Gustafsson LE, Hammer J, Lonnqvist PA, Sanchez-Luna M, Sedin G, Subhedar N: Inhaled nitric oxide therapy in neonates and children: Reaching a European consensus. Intensive Care Med 2004; 30:372–80
246. Tanus-Santos JE, Theodorakis MJ: Is there a place for inhaled nitric oxide in the therapy of acute pulmonary embolism? Am J Respir Med 2002; 1:167–76
247. Weller R: Nitric oxide donors and the skin: Useful therapeutic agents? Clin Sci (Lond) 2003; 105:533–5
248. Vallance P: Nitric oxide: Therapeutic opportunities. Fundam Clin Pharmacol 2003; 17:1–10
249. Baxter FJ, Randall J, Miller JD, Higgins DA, Powles AC, Choi PT: Rescue therapy with inhaled nitric oxide in critically ill patients with severe hypoxemic respiratory failure (brief report). Can J Anaesth 2002; 49:315–8
250. Wang T, El Kebir D, Blaise G: Inhaled nitric oxide in 2003: A review of its mechanisms of action. Can J Anaesth 2003; 50:839–46
251. Cobb JP: Nitric oxide synthase inhibition as therapy for sepsis: A decade of promise. Surg Infect (Larchmt) 2001; 2:93–100
252. Christensen KL, Mulvany MJ: Location of resistance arteries. J Vasc Res 2001; 38:1–12
253. Taylor MS, Bonev AD, Gross TP, Eckman DM, Brayden JE, Bond CT, Adelman JP, Nelson MT: Altered expression of small-conductance Ca2+-activated K+ (SK3) channels modulates arterial tone and blood pressure. Circ Res 2003; 93:124–31
254. Vedrinne C, Tronc F, Martinot S, Robin J, Allevard AM, Vincent M, Lehot JJ, Franck M, Champsaur G: Better preservation of endothelial function and decreased activation of the fetal renin-angiotensin pathway with the use of pulsatile flow during experimental fetal bypass. J Thorac Cardiovasc Surg 2000; 120:770–7
255. Undar A, Masai T, Beyer EA, Goddard-Finegold J, McGarry MC, Fraser CD Jr: Pediatric physiologic pulsatile pump enhances cerebral and renal blood flow during and after cardiopulmonary bypass. Artif Organs 2002; 26:919–23
256. Macha M, Yamazaki K, Gordon LM, Watach MJ, Konishi H, Billiar TR, Borovetz HS, Kormos RL, Griffith BP, Hattler BG: The vasoregulatory role of endothelium derived nitric oxide during pulsatile cardiopulmonary bypass. ASAIO J 1996; 42:M800–4
257. Champsaur G, Vedrinne C, Martinot S, Tronc F, Robin J, Ninet J, Franck M: Flow-induced release of endothelium-derived relaxing factor during pulsatile bypass: Experimental study in the fetal lamb. J Thorac Cardiovasc Surg 1997; 114:738–44
258. Busse R, Fleming I: Pulsatile stretch and shear stress: Physical stimuli determining the production of endothelium-derived relaxing factors. J Vasc Res 1998; 35:73–84
259. Drummond JC: MAC for halothane, enflurane, and isoflurane in the New Zealand white rabbit: And a test for the validity of MAC determinations. Anesthesiology 1985; 62:336–8
260. Orliaguet G, Vivien B, Langeron O, Bouhemad B, Coriat P, Riou B: Minimum alveolar concentration of volatile anesthetics in rats during postnatal maturation. Anesthesiology 2001; 95:734–9
261. Iranami H, Hatano Y, Tsukiyama Y, Yamamoto M, Maeda H, Mizumoto K: Halothane inhibition of acetylcholine-induced relaxation in rat mesenteric artery and aorta. Can J Anesth 1997; 44:1196–203
262. Akata T, Nakashima M, Kodama K, Boyle WA III, Takahashi S: Effects of volatile anesthetics on acetylcholine-induced relaxation in the rabbit mesenteric resistance artery. Anesthesiology 1995; 82:188–204
263. Scheller MS, Saidman LJ, Partridge BL: MAC of sevoflurane in humans and the New Zealand white rabbit. Can J Anaesth 1988; 35:153–6
264. Doorley BM, Waters SJ, Terrell RC, Robinson JL: MAC of I-653 in beagle dogs and New Zealand white rabbits. Anesthesiology 1988; 69:89–91
265. De Wit C, Esser N, Lehr HA, Bolz SS, Pohl U: Pentobarbital-sensitive EDHF comediates ACh-induced arteriolar dilation in the hamster microcirculation. Am J Physiol 1999; 276:H1527–34
266. Horibe M, Ogawa K, Sohn JT, Murray PA: Propofol attenuates acetylcholine-induced pulmonary vasorelaxation: Role of nitric oxide and endothelium-derived hyperpolarizing factors. Anesthesiology 2000; 93:447–55